Université de Montréal

Caractérisation de la composante toxicocinétique du facteur d'ajustement pour la variabilité interindividuelle utilisé en analyse du risque toxicologique

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Résumé

Un facteur d'incertitude de 10 est utilisé par défaut lors de l'élaboration des valeurs toxicologiques de référence en santé environnementale, afin de tenir compte de la variabilité interindividuelle dans la population. La composante toxicocinétique de cette variabilité correspond à $\sqrt{10}$, soit 3,16. Sa validité a auparavant été étudiée sur la base de données pharmaceutiques colligées auprès de diverses populations (adultes, enfants, aînés). Ainsi, il est possible de comparer la valeur de 3,16 au Facteur d'ajustement pour la cinétique humaine (FACH), qui constitue le rapport entre un centile élevé (ex. : 95^{e}) de la distribution de la dose interne dans des sous-groupes présumés sensibles et sa médiane chez l'adulte, ou encore à l'intérieur d'une population générale. Toutefois, les données expérimentales humaines sur les polluants environnementaux sont rares. De plus, ces substances ont généralement des propriétés sensiblement différentes de celles des médicaments. Il est donc difficile de valider, pour les polluants, les estimations faites à partir des données sur les médicaments. Pour résoudre ce problème, la modélisation toxicocinétique à base physiologique (TCBP) a été utilisée pour simuler la variabilité interindividuelle des doses internes lors de l'exposition aux polluants. Cependant, les études réalisées à ce jour n'ont que peu permis d'évaluer l'impact des conditions d'exposition (c.-à-d. voie, durée, intensité), des propriétés physico/biochimiques des polluants, et des caractéristiques de la population exposée sur la valeur du FACH et donc la validité de la valeur par défaut de 3,16. Les travaux de la présente thèse visent à combler ces lacunes.

À l'aide de simulations de Monte-Carlo, un modèle TCBP a d'abord été utilisé pour simuler la variabilité interindividuelle des doses internes (c.-à-d. chez les adultes, ainés, enfants, femmes enceintes) de contaminants de l'eau lors d'une exposition par voie orale, respiratoire, ou cutanée. Dans un deuxième temps, un tel modèle a été utilisé pour simuler cette variabilité lors de l'inhalation de contaminants à intensité et durée variables. Ensuite, un algorithme toxicocinétique à l'équilibre probabiliste a été utilisé pour estimer la variabilité interindividuelle des doses internes lors d'expositions chroniques à des

contaminants hypothétiques aux propriétés physico/biochimiques variables. Ainsi, les propriétés de volatilité, de fraction métabolisée, de voie métabolique empruntée ainsi que de biodisponibilité orale ont fait l'objet d'analyses spécifiques. Finalement, l'impact du référent considéré et des caractéristiques démographiques sur la valeur du FACH lors de l'inhalation chronique a été évalué, en ayant recours également à un algorithme toxicocinétique à l'équilibre. Les distributions de doses internes générées dans les divers scénarios élaborés ont permis de calculer dans chaque cas le FACH selon l'approche décrite plus haut. Cette étude a mis en lumière les divers déterminants de la sensibilité toxicocinétique selon le sous-groupe et la mesure de dose interne considérée. Elle a permis de caractériser les déterminants du FACH et donc les cas où ce dernier dépasse la valeur par défaut de 3,16 (jusqu'à 28,3), observés presqu'uniquement chez les nouveau-nés et en fonction de la substance mère. Cette thèse contribue à améliorer les connaissances dans le domaine de l'analyse du risque toxicologique en caractérisant le FACH selon diverses considérations.

Mots-clés : Analyse du risque, Conditions d'exposition, Doses internes, Facteur d'incertitude, Modélisation toxicocinétique à base physiologique, Simulations de Monte-Carlo, Variabilité interindividuelle, Voies métaboliques

Abstract

A default uncertainty factor of 10 is used in toxicological risk assessment to account for human variability, and the toxicokinetic component of this factor corresponds to a value of √10, or 3,16. The adequacy of this value has been studied in the literature on the basis of pharmaceutical data obtained in various subpopulations (e.g. adults, children, elderly). Indeed, it is possible to compare the default value of 3,16 to the Human Kinetic Adjustment Factor (HKAF), computed as the ratio of an upper percentile value (e.g. 95^{th}) of the distribution of internal dose metrics in presumed sensitive subpopulation to its median in adults, or alternatively an entire population. However, human experimental data on environmental contaminants are sparse. Besides, these chemicals generally exhibit characteristics that are quite different as compared to drugs. As a result, it is difficult to extrapolate, for pollutants, estimates of HKAF that were made using data on drugs. To solve this problem, physiologically-based toxicokinetic (PBTK) modeling has been used to simulate interindividual variability in internal dose metrics following exposure to xenobiotics. However, studies realized to date have not systematically evaluated the impact of the exposure conditions (route, duration and intensity), the physico/biochemical properties of the chemicals, and the characteristics of the exposed population, on the HKAF, and thus the adequacy of the default value. This thesis aims at compensating this lack of knowledge.

First, a probabilistic PBTK model was used to simulate, by means of Monte Carlo simulations, the interindividual variability in internal dose metrics (*i.e.* in adults, children, elerly, pregnant women) following the oral, inhalation or dermal exposure to drinking water contaminants, taken separately. Second, a similar model was used to simulate this variability following inhalation exposures of various durations and intensities to air contaminants. Then, a probabilistic steady-state algorithm was used to estimate interindividual variability in internal dose metrics for chronic exposures to hypothetical

contaminants exhibiting different physico/biochemical properties. These include volatility, the fraction metabolized, the metabolic pathway by which they are biotransformed and oral bioavailability. Finally, the impact of a population's demographic characteristics and the referent considered on the HKAF for chronic inhalation exposure was studied, also using a probabilistic steady-state algorithm. The distributions of internal dose metrics that were generated for every scenario simulated were used to compute the HKAF as described above. This study has pointed out the determinants of the toxicokinetic sensitivity considering a given subpopulation and dose metric. It allowed identifying determinants of the numeric value of the HKAF, thus cases for which it exceeded the default value of 3,16. This happened almost exclusively in neonates and on the basis of the parent compound. Overall, this study has contributed to the field of toxicological risk assessment by characterizing the HKAF as a function of various considerations.

Keywords : Exposure conditions, Interindividual variability, Internal dose, Metabolic pathways, Monte Carlo simulations, Physiologically-based pharmacokinetic modeling, Risk Assessment, Uncertainty factor

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Liste des sigles et abréviations

ADH	Alcool déhydrogénase
AEGL	Acute exposure guideline level
ALDH	Aldéhyde déshydrogénase
BMD	Dose-repère pour un effet significatif
C_{max}	Concentration sanguine maximale
COV	Composés organiques volatils
CYP	Cytochrome P-450
E	Ratio d'extraction hépatique
FACH	Facteur d'ajustement cinétique humain
FASC	Facteur d'ajustement spécifique par substance (« *CSAF* » en anglais)
FDP	Fonction de densité de probabilité
FG	Filtration glomérulaire
FH	Facteur d'incertitude pour l'extrapolation animal → humain
FH-TD	Composante toxicodynamique du facteur d'incertitude pour l'extrapolation animal → humain
FH-TC	Composante toxicocinétique du facteur d'incertitude pour l'extrapolation animal → humain
FI-TD	Composante toxicodynamique du facteur d'incertitude pour la variabilité interindividuelle
FI-TC	Composante toxicocinétique du facteur d'incertitude pour la variabilité interindividuelle
FI	Facteur d'incertitude pour la variabilité interindividuelle
IC 95 %	Intervalle de confiance à 95 %
ICRP	International Commission on Radioprotection

IPCS	International Programme on Chemical Safety
IRIS	Integrated Risk Information System
LOEL	Dose minimale avec effets observés («*lowest observed effect level*» en anglais)
MET	Taux de production de métabolite
NAS	National Academy of Science
NHANES	National Health and Nutrition Examination Survey
NOEL	Dose sans effet observé («*No observed effect level*» en anglais)
NRC	National Research Council
OMS	Organisation mondiale de la Santé
Pb	Coefficient de partage sang:air
PBPK/TK	Pharmaco/Toxicocinétique à base physiologique
PC	Poids corporel
PCE	Tétrachloroéthylène
RfC	Concentration de référence
RfD	Dose de référence orale
SSC	Surface sous la courbe
T	Taille
TCE	Trichloroéthylène
TC	Toxicocinétique
TD	Toxicodynamique
U.S. EPA	Agence de protection de l'environnement des États-Unis
VTR	Valeur toxicologique de référence

À mes enfants, en leur souhaitant le meilleur avenir possible…

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Présentation de la thèse

1 Mise en contexte

1.1 Introduction générale

1.1.1 Les principes de l'évaluation du risque toxicologique

Le paradigme directeur de l'Académie des Sciences des États-Unis (NAS) pour l'analyse du risque toxicologique en santé publique se définit en quatre étapes, soit 1) l'identification du danger, 2) l'évaluation de la relation dose-réponse, 3) l'évaluation de l'exposition et 4) l'estimation du risque (NRC, 1983). Cette dernière étape consiste à comparer l'exposition évaluée à l'étape 3) à des valeurs toxicologiques de référence (VTR), déterminées lors de l'étape 2). En ce qui concerne les substances non cancérigènes, ces VTR (doses de référence orale (RfD) ou concentrations de référence (RfC)) sont déterminées par un processus systématique qui consiste premièrement à identifier une dose repère en fonction d'un effet toxicologique critique. Cette dose repère peut être une dose maximale sans effet (« NOEL » en anglais, pour *No Observed Effect Level*), une dose minimale avec effet (« LOEL », pour *Lowest observed Effect Level*), ou encore une dose repère pour un effet significatif (« BMD », pour *Benchmark dose*). Ces doses peuvent être établies lors d'expérimentations animales en laboratoire ou à partir d'observations faites chez les humains. Une analyse critique des études disponibles permet d'arrêter le choix de la dose repère à utiliser.

Traditionnellement, la détermination d'une VTR requiert de diviser la dose repère par le produit de divers facteurs d'incertitude. Une valeur par défaut est attribuée à ces facteurs qui sont appliqués dans le but de compenser l'incertitude engendrée par l'extrapolation de données mesurées ou évaluées dans des conditions différentes de celles sous-tendant l'établissement de la VTR (Dourson et Stara, 1983; Dourson *et al.*, 1996; U.S. EPA, 2002; Haber, 2007; Ritter *et al.*, 2007). Le Tableau 1-I indique les principaux facteurs d'incertitude généralement utilisés. Les facteurs d'incertitude de 10 concernant l'extrapolation animal/humain et la variabilité interindividuelle découlent de la décomposition, par la NAS, de la valeur originellement proposée par Lehman et Fitzhugh

(1954) pour s'assurer d'une « marge de sécurité de 100 » dans l'établissement des doses journalières admissibles pour les additifs alimentaires (Price *et al.*, 1999). La division de la dose repère par le produit des facteurs appropriés permet de déterminer la RfD ou la RfC pour l'humain. À noter que la U.S. EPA (2002) stipule que ce produit ne devrait pas dépasser 3000, auquel cas il est considéré que les données disponibles ne sont pas suffisantes pour permettre d'élaborer une VTR. L'élaboration des normes en santé environnementale, comme les concentrations maximales dans l'eau potable par exemple (Sidhu, 1991; Ritter *et al.*, 2007), se fait à partir des VTR.

Tableau 1-I : Facteurs d'incertitude appliqués lors de la détermination des valeurs toxicologiques de référence[a)]

Facteur d'incertitude (valeur par défaut)	Objectif poursuivi par l'application du facteur d'incertitude
Variabilité interespèce (10)	Appliqué lorsque la dose repère est déterminée chez l'animal alors que l'on veut déterminer une VTR chez l'humain. La valeur de 10 correspond à la moyenne approximative des rapports de l'activité métabolique animal/humain pour les deux espèces animales les plus couramment utilisées, soit la souris (rapport de 13) et le rat (rapport de 6). Ces valeurs sont calculées sur la base du rapport des poids corporels animal/humain, à la puissance 0,7.
Variabilité interindividuelle[b)] (10)	Appliqué pour compenser l'incertitude engendrée par le recours à des données obtenues chez des individus adultes et/ou ne présentant potentiellement que peu de variabilité interindividuelle (ex. : souches d'animaux de laboratoire), alors que la VTR vise à protéger tous les individus d'une population où la variabilité peut être plus importante (jusqu'à un ordre de grandeur) en raison de la présence d'individus plus sensibles.
Extrapolation LOEL → NOEL (10)	Appliqué lorsque la dose repère consiste en un LOEL alors que l'on élabore une VTR censée protéger la population d'effets délétères, donc qui ne devrait être associée à aucun effet.
Extrapolation sous-chronique → chronique (10)	Appliqué lorsque la dose repère a été déterminée lors d'une étude sous-chronique alors que l'on souhaite élaborer une VTR protégeant des expositions chroniques. La valeur de 10 découle

	de ce que les expérimentations animales sous-chroniques se déroulent typiquement sur des durées de 90 jours, soit environ 10 % de la durée de vie du rat ou de la souris, et de l'application du principe de Haber selon lequel l'effet toxicologique dépend du produit « dose × durée », dont la valeur est constante.
Facteur modifiant (3 ou 10)	Appliqué si l'allure de la courbe dose-réponse est difficile à interpréter, si un potentiel cancérigène de type épigénétique est attribué à la substance ou si un nombre insuffisant d'expérimentations a été réalisé pour évaluer adéquatement le potentiel toxique d'une substance. Ainsi, une analyse complète devrait comporter deux études chroniques chez deux espèces différentes (rongeur et non rongeur), une étude de reproduction multi-générations et deux études sur le développement.

a) : détaillés notamment dans Dourson *et al.* (1996) et Ritter *et al.* (2007).

b) : En vertu du *Food Quality Protection Act (FQPA)* aux États-Unis, un facteur supplémentaire de 10 a été proposé pour tenir compte de la sensibilité pré- et post-natale et du manque de données concernant la susceptibilité particulière des enfants. Toutefois, des analyses subséquentes ont statué que cette sensibilité était généralement déjà considérée dans le facteur d'incertitude interindividuelle. Dans ces cas, le facteur supplémentaire en vertu du FQPA ne serait pas requis (Renwick *et al.*, 2000; Pelekis *et al.*, 2001; Dourson *et al.*, 2002).

1.1.2 Le facteur d'incertitude sur la variabilité interindividuelle

Le facteur appliqué pour compenser l'incertitude engendrée par la variabilité interindividuelle humaine, décrit ci-après par « FI », est un concept soutenu par deux modèles théoriques proposé par Price *et al.* (1999) qui sont complémentaires et non mutuellement exclusifs (Figure 1.1). Le premier est le modèle « population sensible » (« s*ensitive population »*), qui considère la population comme composée de plusieurs sous-groupes à sensibilité variable. Dans ce modèle, le FI vise à compenser le fait que les études ayant servi à déterminer la dose repère n'ont possiblement pas inclus tous les sous-groupes sensibles. Le second est le modèle « échantillon taille finie » (« *finite sample size »*), qui considère la population comme un groupe unique composé d'individus à sensibilité variable. Dans ce modèle, le FI compense le fait que la dose repère déterminée expérimentalement peut ne pas représenter le véritable seuil sans effet de toute la

population puisqu'elle a été déterminée sur la base d'un échantillon fini qui peut ne pas avoir été représentatif de la totalité des individus.

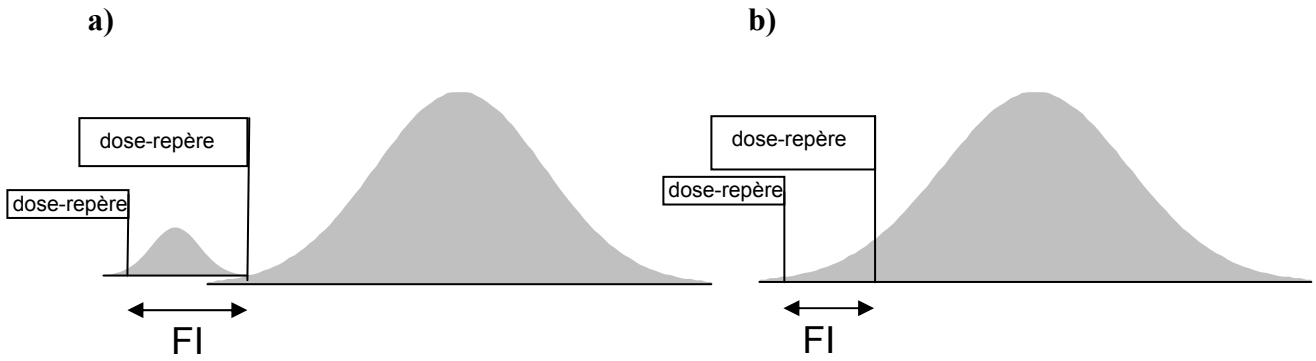


Figure 1.1 : Modèles théoriques décrivant le facteur d'incertitude interindividuelle à partir de la distribution populationnelle de la dose repère (abscisse)-réponse (ordonnée), selon Price *et al.* (1999): a) «population sensible » et b) « échantillon taille finie »

Selon ces deux modèles, le FI vise donc à obtenir une dose « toxicologiquement équivalente » pour les individus présumés sensibles en prenant en considération la variabilité présumée, mais non connue, dans les paramètres toxicocinétiques et toxicodynamiques d'une population (Dourson *et al.*, 1996). Cette variabilité est observée entre les individus des divers sous-groupes composant la population (adultes, enfants, femmes enceintes, personnes âgées, etc.), mais également entre les individus composant un même sous-groupe (Hattis *et al.*, 1999a). La variabilité toxicocinétique concerne les caractéristiques physiologiques influençant les voies d'absorption, de distribution, de transformation et d'élimination d'une substance donnée. La variabilité toxicodynamique concerne le seuil d'apparition des effets néfastes pour chaque individu. La valeur de 10

attribuée implique donc que la dose générant un effet précis chez divers individus d'une population peut varier par un ordre de grandeur.

À partir de données obtenues pour des substances pharmaceutiques chez des sujets humains, Renwick (1993) a pour la première fois proposé de considérer séparément les deux composantes, toxicocinétique et toxicodynamique, du FI et de leur attribuer à chacune une fraction de la valeur par défaut de 10. Dans la même veine, un panel de l'IPCS a conclu que contrairement au cas du facteur d'extrapolation animal→humain (FH) pour lequel des valeurs de 4,0 et 2,5 ont été attribuées respectivement, les données disponibles ne justifiaient pas l'attribution de valeurs différentes à celles-ci dans le cas du FI. Par conséquent, la valeur de 3,16 (√10) a été retenue pour chaque composante (IPCS, 1994), tel qu'indiqué à la Figure 1.2. Cette attribution a été confirmée par les données cinétiques (surface sous la courbe « concentration *vs* temps » (SSC), clairance) et dynamiques analysées par Renwick et Lazarus (1998) sur respectivement 60 et 49 substances pharmaceutiques.

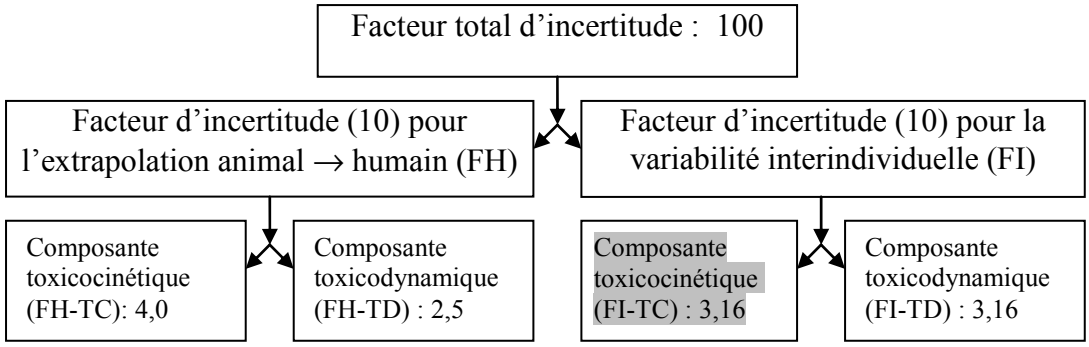


Figure 1.2 : Attribution, aux composantes toxicocinétique (TC) et toxicodynamique (TD), d'une portion de la valeur par défaut de 10 pour les facteurs d'incertitude FH et FI. L'objet d'étude de la présente thèse est indiqué en grisé.

De manière générale, on note depuis plusieurs années dans la littérature scientifique un intérêt envers la quantification du FI et la détermination de sa capacité à protéger adéquatement tous les individus d'une population. Ainsi, Calabrese (1985) a estimé que la valeur de 10 attribuée par défaut était protectrice d'environ 80 à 95 % de la population. Hattis *et al.* (1987) ont pour leur part estimé que 96 % des individus étaient protégés sur la base de plus d'une centaine de groupes de données sur diverses substances, notamment pharmaceutiques. Renwick et Lazarus (1998) ont évalué que la proportion de la population que le facteur de 3,16 toxicocinétique ne couvrirait pas serait en moyenne de moins de 1 % ou 10 %, sur la base de distributions de paramètres pharmacocinétiques assumées comme normales et lognormales, respectivement. Par conséquent, et en tenant compte également de la variabilité toxicodynamique dans une population, un facteur supérieur à la valeur de 10 pourrait être requis pour protéger un pourcentage suffisamment élevé d'individus afin que la probabilité d'apparition d'effets toxiques soit considérée comme négligeable (Hattis *et al.*, 1999b). D'autre part, Dourson *et al.* (2002) font état qu'une réduction du facteur de 10 puisse être considérée appropriée si suffisamment de données suggèrent que la présence de sous-groupes sensibles est improbable ou si la dose repère a déjà été déterminée dans un sous-groupe sensible. La valeur de 1 est attribuée au FI dans ce dernier cas.

Dans la section 1.2 qui suit, les différences physiologiques entre les adultes et les autres sous-groupes de la population pouvant être à l'origine d'une susceptibilité accrue de ces derniers sont d'abord décrites. Par la suite, les études ayant porté sur la quantification de cette susceptibilité sont présentées. Cela a permis de cerner la problématique étudiée dans la présente thèse (section 1.3) et de formuler les objectifs en découlant (section 1.4).

1.2 État des connaissances

1.2.1 Résumé des différences dans les paramètres physiologiques entre les divers sous-groupes de la population et l'adulte

Il est bien connu que les paramètres physiologiques influençant la toxicocinétique des substances peuvent être différents entre l'adulte et les divers sous-groupes de la population. Ces variations font l'objet de la présente section et sont décrites brièvement pour les trois principaux sous-groupes, soit la femme enceinte, les enfants et les aînés (Tableau 1-II). Des variations dans la toxicocinétique des substances sont également observées entre les hommes et les femmes à tous les âges (Krishnan et Andersen, 1993), mais particulièrement à l'âge adulte. Les principales différences consistent en une diminution chez la femme du taux d'inhalation moyen, en une augmentation de la proportion corporelle de tissus adipeux, ainsi qu'en une diminution de la masse du corps et des organes (Brown *et al.*, 1998). Certaines enzymes de biotransformation seraient plus élevées chez l'homme que chez la femme. De plus, les femmes qui allaitent ont la possibilité d'excréter les substances fortement lipophiles par le lait maternel (Clewell *et al.*, 2002).

Tableau 1-II : Résumé des différences dans les diverses étapes de la toxicocinétique humaine entre l'adulte et d'autres sous-groupes présumés sensibles de la population

Étapes de la toxicocinétique	Sous-groupe		
	Nouveau-nés/enfants[a)]	Femmes enceintes[b)]	Aînés[a)]
Absorption			
Orale	↑↓	↑↓	↑↓
Inhalation	↑	↑	I
Cutanée	I	↑	↓
Distribution (substances)			
Lipophiles	↑	↑	↑
Hydrophiles	↑	↑	↓
Liées aux protéines plasmatiques	↓	↑↓	↓
Métabolisme (enzymes)	↓ [c)]	↑↓	I
Fonction rénale[d)]	↓	↑	↓

↑: Plus élevé que l'adulte moyen; ↓ Plus faible que l'adulte moyen ↑↓: Augmentation ou diminution, selon les substances; I: Données insuffisantes pour trancher ou aucune différence confirmée à ce jour.

a) : Valcke et Krishnan (2009), Clewell *et al.* (2002).

b) : Krauer *et al.* (1980), Mattison (1990), Faustman et Ribeiro (1990) et Mattison *et al.* (1991).

c) : Sauf dans le cas des sulfotransférases, qui semblent plus élevées chez les jeunes enfants que chez l'adulte.

d) : Sur la base de la filtration glomérulaire et de la sécrétion tubulaire.

1.2.1.1 Femmes enceintes

Chez la femme enceinte, des taux augmentés d'ingestion d'aliments et d'eau ainsi qu'une augmentation de la surface cutanée et du taux de ventilation alvéolaire (près de 50 % dans ce dernier cas) peuvent contribuer à une augmentation de l'exposition aux xénobiotiques et des échanges gazeux (Mattison *et al.*, 1991; IPCS, 2006). Le temps de vidange gastrique est augmenté alors que la motilité intestinale diminue, ce qui résulte en une rétention plus longue des aliments. Le volume d'eau et les débits sanguins cardiaque et périphériques sont

augmentés d'environ 30 % durant le premier trimestre et le volume plasmatique augmente de près de 50 % (Krauer *et al.*, 1980). Les variations dans les concentrations de protéines plasmatiques (augmentation des globulines et diminution de l'albumine, par près de 40 % dans ce dernier cas) influencent aussi le volume de distribution des xénobiotiques (Krauer *et al.*, 1980; Faustman et Ribeiro, 1990). Ce dernier se définit comme la somme du volume de sang et des produits des volumes de chaque tissu multiplié par leurs coefficients de partition tissu: sang respectifs. Pour ce qui est de la clairance, certaines variations peuvent être observées au niveau de l'activité métabolique relative au contenu en enzymes de biotransformation (Mattison *et al.*, 1991). Toutefois, puisque le débit sanguin absolu au foie ne varie pas (ICRP, 2002), la proportion du débit cardiaque affectée à la perfusion hépatique diminue. Finalement, la perfusion rénale et le taux de filtration glomérulaire sont augmentés d'environ 30 % chez la femme enceinte (Krauer *et al.*, 1980; Faustman et Ribeiro, 1990).

1.2.1.2 Enfants

Bien qu'il soit généralement admis qu'il existe des différences importantes dans les caractéristiques physiologiques et pharmacocinétiques entre les enfants à tous les stades de l'enfance et les adultes, ces différences sont beaucoup plus significatives dans la première année de vie (Renwick *et al.*, 2000; Ginsberg *et al.*, 2004a). Ainsi, tel que le montre le Tableau 1-.III, la surface cutanée ainsi que les taux d'inhalation et d'ingestion d'eau et d'aliments sont plus élevés chez les enfants que chez l'adulte, lorsqu'ajustés au poids corporel (NRC, 1993). La perméabilité cutanée est également accrue. De plus, certains comportements spécifiques aux jeunes enfants, comme l'ingestion de sol et le contact main-bouche, constituent des sources supplémentaires d'exposition en comparaison de l'adulte (Reed *et al.*, 1999; Miller *et al.*, 2002). Chez les nouveau-nés, l'exposition par l'ingestion de lait maternel peut être significative (Jensen, 1991; Fisher *et al.*, 1997), particulièrement dans le cas des substances fortement liposolubles. Ainsi, l'ampleur du transfert des contaminants présents dans le lait maternel dépend des propriétés physico-chimiques de la substance et de la physiologie de la mère.

Tableau 1-III : Comparaison des taux d'exposition par diverses voies chez des enfants de divers âges par rapport à l'adulte[a)]

Age	Poids corporel[b)]	Taille[b)]	Taux d'ingestion d'eau potable[c)]	Taux d'ingestion d'aliments[d)]	Taux d'inhalation[e)]	Surface cutanée[e)]
ans	kg	cm	L/kg-d	g/kg-d	m^3/d (/kg)	Cm^2 (/kg)
1	8,6	74,6	0,035	10,6	1,9 (0,22)	4390 (510)
3	15,0	94,4	0,046	8,1	2,9 (0,19)	6451 (430)
6	22,2	117,8	0,036	6,7	4,0 (0,18)	8672 (390)
30	71,8	168,5	0,019	4,1	9,8 (0,14)	18 462 (257)

a) : Modifié de Valcke et Krishnan (2009).

b) : Valeurs moyennes pour les deux sexes, calculées à l'aide des équations de Haddad *et al.* (2001).

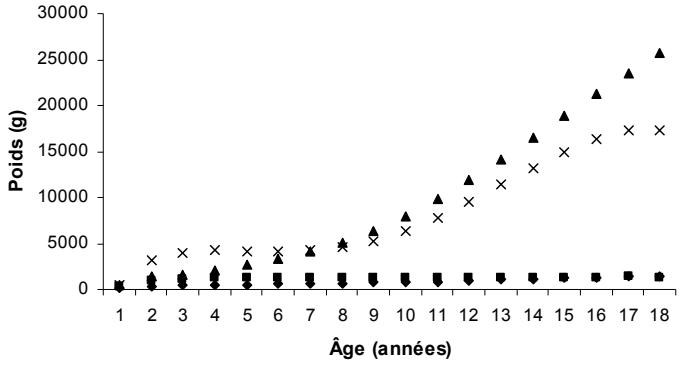
c) : Calculés en utilisant le taux d'ingestion moyen (L/d), tel que décrit par la U.S. EPA (1997a), divisé par le poids corporel. Le taux d'ingestion des enfants de 1 an est plus bas puisque plusieurs sont allaités.

d) : Exemple pour la viande; taux calculés en utilisant les valeurs de la U.S. EPA (1997a) divisées par le poids corporel.

e) : Calculées à l'aide des équations décrites par Haddad *et al.* (2006).

Les volumes tissulaires normalisés au poids corporel varient en fonction de l'âge (Jacquz-Aigrain, 2001; Clewell *et al.*, 2002; Price *et al.*, 2003a), mais les variations dans les taux de perfusion des organes et tissus ne suivent pas les mêmes tendances (Figure 1.3).

a)



b)

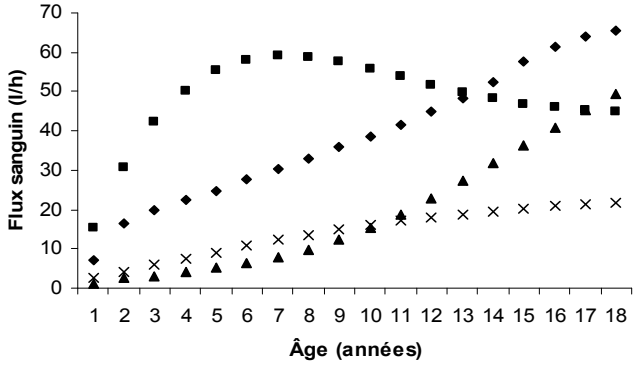


Figure 1.3 : Variation dépendante de l'âge du poids (a) et de la perfusion (b) du foie (♦), du cerveau (■), des muscles (▲) et des tissus adipeux (×), chez l'humain (Tiré de Valcke et Krishnan, 2009). Les poids sont calculés d'après les équations de Haddad *et al.* (2001). Les flux sanguins sont calculés tels que décrits par Price *et al.* (2003a).

Bien que des différences dans le contenu en lipide et en eau des organes soient observées en fonction de l'âge (IPCS, 2006), les coefficients de partage tissus:sang et sang:air ne montrent pas de variations importantes (Mahle *et al.*, 2007; Malviya et Lerman, 1990). Les concentrations en protéines sériques semblent stables avec l'âge, exception faite de

l'albumine qui est moins présente chez les nouveau-nés que chez les adultes (Alcorn et McNamara, 2002; Clewell *et al.*, 2002).

Quoique les niveaux d'activité des enzymes de biotransformation de Phase I soient réduits à la naissance, ils se rapprochent presque tous des niveaux adultes vers la fin de la première année de vie (Hakkola *et al.*, 1998). Les CYP1A2 constituent des exceptions, puisque l'atteinte des niveaux adultes semble s'échelonner sur plusieurs années. Dans certains cas toutefois, de jeunes enfants peuvent présenter des niveaux d'enzymes supérieurs à ceux de l'adulte (Cresteil, 1998; Johnsrud *et al.*, 2003). Pour ce qui est des enzymes de Phase II, la capacité de conjugaison à la glycine et de sulfatation chez les jeunes enfants sont comparables aux adultes, alors que la glucuronidation ainsi que la conjugaison au glutathion sont réduites (IPCS, 2006).

La proportion du débit cardiaque qui est dirigée vers le système rénal est réduite chez le nouveau-né en comparaison de l'adulte (IPCS, 2006). La filtration glomérulaire et les phénomènes de sécrétion et de réabsorption tubulaire sont réduits chez l'enfant (Renwick, 1998). Le tout résulte en une clairance rénale diminuée par un facteur variant entre 30 % et 50 % par rapport à l'adulte (Clewell *et al.*, 2002; Kearns *et al.*, 2003). La filtration glomérulaire absolue prend plusieurs années à atteindre les niveaux adultes (Sarangapani *et al.*, 2003; DeWoskin et Thompson, 2008), comparativement à quelque mois, lorsque normalisée sur la base de la surface corporelle (127 ml/min-1,73 m^2). Les fonctions tubulaires sont matures vers l'âge de 1 an (Crom, 1994; Jacquz-Aigrain, 2001; Alcorn et McNamara, 2003; DeWoskin et Thompson, 2008).

Enfin, il apparaît pertinent de noter que la majorité des différences entre les nouveau-nés et les adultes sont d'autant accentuées si on considère le nouveau-né prématuré et *a fortiori* le

fœtus, qui est un organisme en développement. Ses systèmes physiologiques sont donc particulièrement immatures (Green *et al.*, 1979; Finster et Pedersen, 1979; Mattison, 1990; Faustmann et Ribeiro, 1990; Mattison *et al.*, 1991). Des différences dans les propriétés fonctionnelles, comme l'immaturité de la barrière hémo-encéphalique protégeant le cerveau des substances xénobiotiques (Gray, 1995), sont également observées. L'exposition fœtale a lieu via le placenta, que toutes les substances franchissent à des degrés variables selon leurs propriétés physico-chimiques.

1.2.1.3 Ainés

Les aînés représentent une portion de plus en plus importante des populations en Occident et imposent une pression accrue sur les systèmes de santé, ceci en raison des incidences plus élevées de maladie qu'on retrouve chez ce sous-groupe par rapport aux sous-groupes plus jeunes (Schmucker et Lonergan, 1987). Ces incidences plus élevées augmentent d'ailleurs la variabilité dans la pharmacocinétique des substances chez les aînés (Groen *et al.*, 1993; Kinirons et Crome, 1997). Comparés à l'adulte moyen, une diminution des niveaux de mobilité et d'activité physique chez les aînés entraîne une diminution de la consommation d'eau et d'aliments. Des différences sont également observées au niveau de la physiologie gastro-intestinale, notamment une réduction de la motilité gastrique. Une atrophie de la vascularisation cutanée ainsi qu'une hydratation réduite de la peau sont observées et peuvent se traduire par une absorption cutanée réduite des xénobiotiques. Des changements structuraux au niveau pulmonaire (diminution de l'élasticité, réduction de la surface alvéolaire) résultent en une diminution des échanges gazeux (Clewell *et al.*, 2002).

L'adulte sénescent montre une diminution de la masse musculaire et du contenu en eau ainsi qu'une augmentation de la proportion de graisses et une diminution (modeste) de la concentration plasmatique d'albumine. Cette dernière réduction peut être plus importante chez les individus atteints de diverses maladies (cirrhose, insuffisance rénale, arthrite, etc.,

Dawling et Crome, 1989). L'inverse est observé pour la globuline (Clewell *et al.*, 2002). Un débit cardiaque diminué de 1 % par année après l'âge de 30 ans résulte en une diminution de la perfusion tissulaire (Dawling et Crome, 1989).

Les principales différences physiologiques relatives à la clairance chez l'adulte sénescent en comparaison de l'adulte moyen sont une perfusion et un volume hépatique réduit. Une réduction de la capacité des systèmes de transport hépatobiliaires limite aussi la capacité de biotransformation du foie (Schmucker et Lonergan, 1987; Schmucker, 2001; Zeeh et Platt, 2002). La diminution de la fonction rénale, par un facteur pouvant atteindre environ 65 %, joue également un rôle important dans la diminution de la clairance systémique. En effet, la filtration glomérulaire et la sécrétion tubulaire diminuent d'environ 0,6 % par année à partir de 30 ans, alors que la masse et la perfusion rénale peuvent diminuer par près de 50 % (Clewell *et al.*, 2002).

1.2.2 Détermination de la valeur de la composante toxicocinétique du facteur d'ajustement interindividuel

1.2.2.1 Principe général

Dans un document d'orientation publié en 2005, l'Organisation mondiale de la Santé (OMS) encourage, dans la mesure du possible, le recours aux données quantitatives disponibles pour déterminer la valeur réelle des facteurs FH et FI afin de diminuer le recours aux approches par défaut, empreintes d'incertitude, dans l'évaluation du risque (IPCS, 2005). Selon Haber (2007), la valeur attribuée à chaque composante décrite à la Figure 1.2 dépend de la disponibilité des données et suit donc un continuum « données absentes $\Rightarrow$ données nombreuses », comme illustré à la Figure 1.4. Cette approche assure une protection que l'on peut qualifier comme allant de « présumée » à « orientée sur la

biologie » (Meek *et al.*, 2002a). Ainsi, la valeur par défaut est attribuée en **l'absence de toute donnée**. À l'opposé, quand un facteur peut être estimé à partir de **nombreuses données concernant des substances spécifiques,** un facteur d'ajustement spécifique par substance chimique (**FASC,** « *CSAF* » en anglais) peut être déterminé (IPCS, 2005). À cette fin, les données disponibles doivent porter autant sur les mécanismes d'action impliqués que sur la variabilité de cette mesure chez l'animal (si requis) et l'humain. La connaissance des mécanismes d'action inclut la connaissance de l'entité toxique responsable de l'effet sur lequel se base l'élaboration de la VTR de même que la mesure de dose interne y étant le mieux associée (ex. : concentration sanguine maximale (C_{max}), taux de production de métabolites (MET), SSC). Des facteurs différents de la valeur par défaut peuvent aussi être déterminés en se basant sur des **données générales spécifiques ou non aux substances** sans toutefois que les mécanismes d'action toxiques aient été élucidés de manière satisfaisante. On parle alors de facteurs dits « basés sur des données » (« *data-derived* » en anglais). La détermination de la composante toxicocinétique du FH par l'ajustement du taux de métabolisme en fonction du rapport des poids corporels à la puissance 0,75 en est un exemple (Lipscomb, 2008). Considérer la variabilité de paramètres pharmacocinétiques pour des substances partageant la même voie métabolique en est un autre (Renwick *et al.*, 2001). À l'occasion, cette approche peut servir à déterminer des facteurs dits « catégoriels », c.-à-d. pour des catégories de substances regroupées selon certaines similarités physico/biochimiques (Naumann *et al.*, 2001). Toutefois, ceci se fait au coût d'une incertitude introduite justement par la généralisation à plusieurs substances non étudiées de propriétés observées pour quelques substances d'une « catégorie » définie.

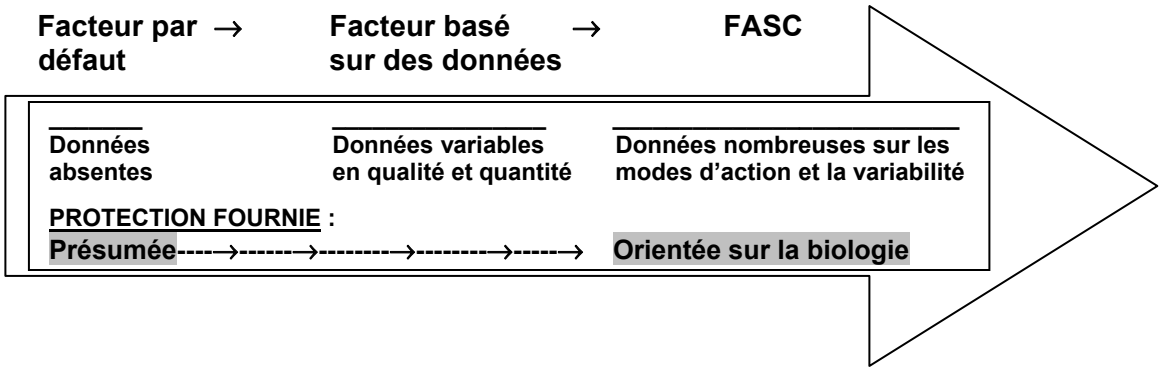


Figure 1.4 : Continuum de la méthode de détermination du FH et du FI en fonction des données disponibles

Le facteur d'ajustement « composite » final, lequel remplace le facteur total d'incertitude par défaut de 100 (Figure 1.2), découle de la multiplication des valeurs déterminées selon ce continuum pour chaque composante toxicocinétique et toxicodynamique du FH et du FI (Meek *et al.*, 2002b; IPCS, 2005). Une telle approche a été utilisée jusqu'à maintenant pour cinq substances dans le cadre du programme IRIS de la U.S. EPA, soit l'éthylène glycol-butyl-éther, le chlorure de vinyle, le dichlorométhane, le xylène et le bore (DeWoskin *et al.*, 2007). Grant *et al.* (2010) pour le 1,3-butadiène, de même que Palmer et Brent (2005), pour l'éthylène glycol, ont également utilisé cette approche pour proposer des facteurs différents des valeurs par défaut. À noter que la valeur par défaut de la composante toxicocinétique du facteur d'incertitude interindividuelle n'a été remplacée par une valeur différente (2) que dans le cas du bore. Pour la suite de la présente thèse, cette composante toxicocinétique, lorsque différente de la valeur par défaut et déterminée sur la base de données modélisées ou non, sera appelée «facteur d'ajustement pour la cinétique humaine » ou « FACH ». Ce terme réfère à l'expression anglaise *human kinetic adjustment factor* (ou *HKAF*), utilisée dans les articles.

1.2.2.2 Recours à des données expérimentales

L'attribution d'un FACH peut se faire sur la base de mesures expérimentales sur les paramètres d'intérêt (ex. : SSC, C_{max}) ou encore sur les déterminants physiologiques de ces paramètres (ex. : filtration glomérulaire, clairance hépatique). Dans ce contexte, l'approche proposée par l'OMS requiert de définir d'abord une distribution populationnelle des valeurs relatives aux paramètres toxicocinétiques concernant l'entité toxique d'intérêt (ex. : SSC ou C_{max} pour la substance mère, MET pour les métabolites) ou à leurs déterminants (IPCS 2005). Puis, la détermination du FACH se fait en calculant le rapport entre 1) un centile élevé ou faible selon celui qui est associé à une susceptibilité accrue (ex. : 5^{e} de la clairance ou 95^{e} de la demi-vie) et 2) la médiane de la distribution du paramètre concerné (Figure 1.5a). Par exemple, si on considère la clairance, la valeur sera donnée par le rapport [médiane/5^{e} centile]. Si l'on considère la concentration sanguine, la valeur sera donnée par le rapport [95^{e} centile/médiane]. La valeur prise par ces centiles peut être mesurée ou calculée à partir de la forme assumée de la distribution (ex. : lognormale) et de ses paramètres statistiques (ex. : moyenne, écart-type).

Si des évidences existent quant à la présence de sous-groupes particulièrement sensibles à l'intérieur de cette population générale (ex. : sujets présentant un polymorphisme, nouveau-nés, personnes âgées ou malades, etc.), une distribution bimodale peut être envisagée. Dans ce cas, le calcul du FACH se fait en calculant le rapport du centile extrême du sous-groupe sensible, sur la médiane de la population en général (Figure 1.5b). Cette procédure apparaît particulièrement importante dans le cas de l'évaluation de risque pour des sous-groupes particuliers, puisque par exemple, le 95^{e} centile d'un sous-groupe le plus sensible de la population générale, mais ne représentant que 1 % de celle-ci, correspond en fait au 99,95^{e} centile de la population en général. Par conséquent, si une distribution unimodale est choisie pour déterminer le FACH de cette population, ces individus ne seraient pas couverts même en considérant le rapport entre le 99^{e} centile et la médiane de la population (Dorne et Renwick, 2005).

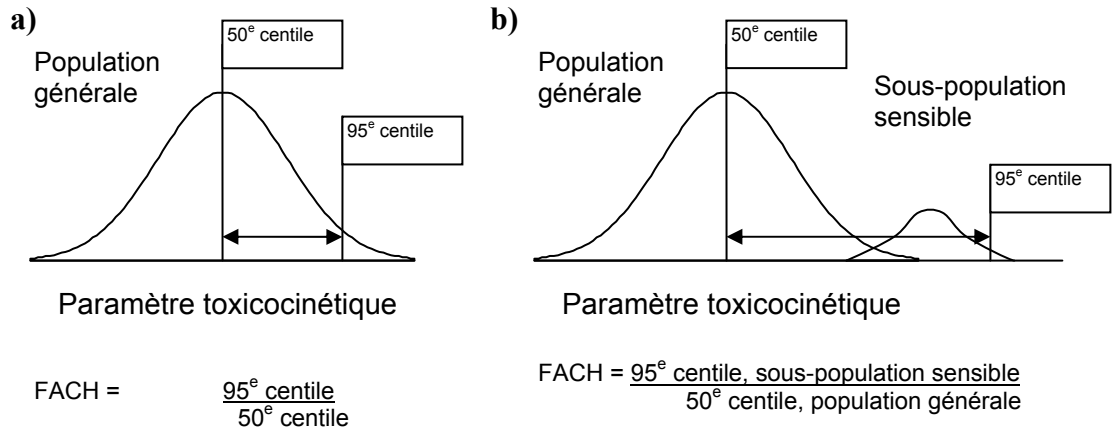


Figure 1.5 : Méthode de calcul du FACH basée sur une distribution unimodale (a) ou bimodale (b) de paramètres toxicocinétiques dans des populations données

À l'exception du bore et de l'acétaldéhyde, un métabolite de l'éthanol, l'évaluation de la variabilité populationnelle pharmacocinétique à partir de mesures expérimentales n'a porté que sur des substances médicamenteuses (ex. : Silvermann *et al.*, 1999; Skowronski et Abdel-Rahman, 2001; Suh et Abdel-Rahman, 2002; Ginsberg *et al.*, 2002a; Hattis *et al.*, 2003; Naumann *et al.*, 2001; 2004; Dorne *et al.*, 2005). À cet égard, les études de Ginsberg *et al.* (2002b), de Hattis *et al.* (2003), et de Dorne *et al.* (2005) sont particulièrement intéressantes, car elles mettent en relation la variabilité populationnelle de la pharmacocinétique des substances en fonction de la voie qu'elles empruntent lors de leur biotransformation ou de leur élimination. Elles seront donc décrites un peu plus loin.

Mais d'abord, en ce qui a trait aux substances non médicamenteuses, Zhao *et al.* (1999) rapportent le cheminement suivi par le *Working Group on Chemical Substances in Drinking Water*, de l'OMS, qui s'est basé sur la variabilité de la filtration glomérulaire observée chez les femmes enceintes pour proposer un FACH de 1,8 pour le bore. Cette attribution se base sur le fait que 100 % du bore absorbé est éliminé dans l'urine sous forme

inchangée, ainsi que sur la base d'une variabilité maximale assumée de deux écarts-types sous la moyenne, pour la filtration glomérulaire chez la femme enceinte. Cette variabilité était déterminée en fonction de la plus petite valeur pouvant être observée tout en permettant la survie. La U.S. EPA a repris l'exercice dans le cadre du programme IRIS et a analysé une plus grande quantité de données, tout en utilisant comme repère de variabilité une étendue de trois écarts-types sous la moyenne afin de couvrir un plus grand écart de valeurs. La valeur moyenne de FACH résultante était de 2 (DeWoskin *et al.*, 2007).

Par ailleurs, en se référant à des données expérimentales sur la concentration sanguine en acétaldéhyde suite à une ingestion d'éthanol pour des sujets appartenant à diverses ethnies et présentant divers phénotypes pour l'aldéhyde déhydrogénase-2, Ginsberg *et al.* (2002a) ont eu recours à des simulations de Monte-Carlo (voir plus loin) pour simuler la variabilité populationnelle dans les concentrations sanguines de cette substance. Ils ont ainsi généré des données faisant état d'un FACH de 14 si l'on se base sur le 95^{e} centile de la distribution obtenue chez la sous-population asiatique, par rapport à la médiane de la population de l'ensemble des États-Unis.

En ce qui concerne les études sur les substances médicamenteuses mentionnées plus haut, Hattis *et al.* (2003) ont eu recours à une base de données ayant permis à Ginsberg *et al.* (2002b) d'étudier les rapports des demi-vies de 45 substances pharmaceutiques entre l'adulte et les enfants de divers âges. Ils ont ainsi suggéré des FACHs « semi-spécifiques » propres à des catégories de substances qui partagent la même voie métabolique. Ces auteurs ont en effet calculé des facteurs de variabilité interindividuelle entre l'adulte et les autres groupes d'âge, sur la base des valeurs moyennes des demi-vies des substances classées selon leur voie d'élimination et/ou de biotransformation principale. Pour les huit substances excrétées inchangées au niveau rénal, les données suggèrent une valeur maximale moyenne (IC 95 %) de 2,78 (1,4–5,4), pour le rapport entre l'adulte et l'enfant à la naissance. Après deux mois de vie, le rapport tourne autour de 1, indiquant l'absence de différence entre les

adultes et les enfants. Pour les 18 substances éliminées par la voie des cytochromes P-450, un facteur moyen de 4,52 (écart : 2,5–8), 1,83 (écart : 1,4–2,3) et 3,51 (écart : 3,1–4) devrait être appliqué pour protéger respectivement les prématurés, les enfants de un à sept jours et les enfants d'une semaine à deux mois. Toutefois, en considérant spécifiquement la voie d'élimination par le CYP1A2, la différence moyenne de demi-vie pour la théophylline et la caféine entre les adultes et les nouveau-nés à terme sous-tendrait un FACH de 9,45 (écart : 2,9–31). Pour ce qui est des six substances étant biotransformées principalement par la voie de la glucuronidation directement, le rapport observé était de 4,4 (écart : 4,1–4,7) pour les prématurés, de 2,98 (écart : 2,8–3,2) pour les enfants nés à terme et de 2,15 (écart : 1,7–2,7) pour les enfants jusqu'à deux mois d'âge, après quoi les valeurs obtenues se rapprochaient de 1.

Dorne *et al.* (2005) ont décrit l'examen de données pharmacocinétiques (clairance rénale, SSC, C_{max}) suite à l'exposition orale ou intraveineuse de substances dont la biotransformation passaient majoritairement (> 60 %) par l'excrétion rénale sous forme inchangée, ainsi que par les réactions enzymatiques de phase I (ex. : médiée par les cytochromes P450, par l'ADH, ou par hydrolyse) ou II (ex. : glucuronidation, conjugaison à la glycine, sulfatation, acétylation). Ils ont aussi pris en considération les différences de phénotype pour les enzymes présentant un polymorphisme ainsi que les différences interethniques. Les résultats sont présentés au Tableau 1-IV et montrent que la valeur par défaut de 3,16 était souvent insuffisante pour protéger les nouveau-nés, et ce, même quand les enzymes concernées ne présentaient pas de polymorphisme. C'était aussi le cas pour le CYP3A4 chez les aînés. Pour les enzymes sujettes au polymorphisme, la valeur par défaut de 3,16 était insuffisante pour couvrir les adultes présentant le polymorphisme sensible, particulièrement dans le cas du CYP2C19 et 2D6. Pour ces deux enzymes, des différences importantes reliées à l'ethnicité étaient également observées, de même que pour les substances transformées par le CYP3A4, par hydrolyse et par acétylation. Pour les substances excrétées majoritairement sous forme inchangée dans l'urine, la valeur par

défaut de 3,16 protégeait plus de 95 % des adultes et des sous-groupes, sauf dans le cas des aînés, pour lesquels un FACH de 3,3 était requis pour avoir le même niveau de protection.

Tableau 1-IV : FACHs catégoriels déterminés dans la littérature, selon la voie métabolique empruntée[a)]

Voie métabolique	FACHs[b)] catégoriels pour divers sous-groupes				
	Adulte (16–70 ans)	Nouveau-nés (< 1 mois)	Enfants (1–16 ans)	Aînés (> 70 ans)	Ethnies[c)]
Phase I					
Monomorphiques					
CYP1A2	1,6	11	1,4	1,4	2,5
CYP2A6	1,6	nd	nd	1,9	2,2
CYP2E1	1,5	nd	nd	1,9	nd
CYP3A4	2,1	8,1	1,4	3,6	5,7
ADH	1,5	nd	nd	2,4	1,2
Hydrolyse	1,6	nd	nd	1,2	3,8
Polymorphiques[d)]					
CYP2C9	2,1	nd	nd	1,2	2,3
CYP2C19	45	nd	5,4	nd	24
CYP2D6	21	nd	22	5	8,2
Phase II					
Monomorphiques					
Glucuronidation	1,6	8,6	1,3	2,3	nd
Gly-conjugaison	1,4	25	1,5	1,6	nd
Sulfatation	1,5	nd	nd	1,6	1,2
Polymorphiques[d)]					
Acétylation	4,4	nd	2,2	6,3	5,2
Clairance rénale	1,4	2,8	1,2	3,3	1,2

nd : non disponible. Les surimpressions grisées indiquent les dépassements de la valeur par défaut de 3,16.

a) : Adapté de Dorne *et al.* (2005).

b) : Calculé comme étant le rapport du 95^{e} centile de la clairance ou de la SSC du sous-groupe sur la valeur moyenne chez l'adulte. Lorsque les données étaient disponibles pour les deux paramètres, la valeur moyenne de FACH pour les deux mesures était calculée.

c) : La valeur du FACH indiquée correspond à la plus élevée parmi les diverses ethnies considérées.

d) : La valeur du FACH indiquée correspond à la plus élevée parmi les divers polymorphismes étudiés.

1.2.2.3 Recours à des données modélisées

Puisque les données expérimentales sur les contaminants environnementaux se font rares, mises à part celles, quand même peu répandues, colligées chez des individus adultes en bonne santé, l'évaluation de la variabilité des doses internes à ces substances a nécessité le recours, par la communauté scientifique, à la modélisation toxicocinétique à base physiologique (TCBP). En effet, cette approche permet de simuler, à l'aide d'équations différentielles, les doses internes (ex. : C_{max}, SSC, MET) suite à l'exposition aux contaminants environnementaux. Pour ce faire, cette approche implique d'utiliser, comme paramètre d'entrée des modèles, les données physiologiques et physico-chimiques telles que les volumes tissulaires, débits sanguins, coefficients de partage, clairance intrinsèque, etc. (Krishnan et Andersen, 2008), en plus bien sûr de la dose ou de la concentration d'exposition. Pour des expositions chroniques continues à faibles doses, on peut considérer qu'un état d'équilibre est éventuellement atteint et qu'alors, tout apport de polluant dans l'organisme est compensé par l'élimination d'une quantité équivalente. Dans cet état, la concentration sanguine de polluant et la quantité de métabolites produits par unité de temps ne varient plus. Lorsque l'exposition est très inférieure à celle entraînant la saturation métabolique, il est possible de calculer ces mesures de doses internes par des équations simples non différentielles. Celles-ci présentent l'avantage de donner des résultats très comparables aux modèles TCBP complexes, mais en requérant beaucoup moins de paramètres (Pelekis *et al.*, 1997).

La modélisation TCBP repose souvent sur le recours à des valeurs moyennes uniques pour les paramètres du modèle, provenant de la littérature ou de diverses bases de données (ex. : Arms et Travis, 1988; U.S. EPA, 1997a; ICRP, 2002). Ces valeurs peuvent aussi être calculées à partir de l'âge, de la taille ou du poids corporel (ex. : Haddad *et al.*, 2001; Price *et al.*, 2003a; 2003b; Clewell *et al.*, 2004;, Beaudoin *et al.*, 2010). On parle dans ces cas de modélisation « déterministe », laquelle ne prend donc pas en considération la variabilité interindividuelle retrouvée pour chaque paramètre et pour chaque âge. La méthode

« probabiliste » de simulations de Monte-Carlo permet de combler cette lacune (U.S. EPA, 1997b). En effet, elle considère la variabilité propre à chaque paramètre d'entrée sous la forme de fonctions de densité de probabilités (FDP), lesquelles peuvent être spécifiées en fonction de distributions de valeurs retrouvées dans la littérature (ex. : Price *et al.*, 2003b). Puis, lors de simulations, le modèle est résolu dans un processus itératif en prélevant, à chaque répétition, les valeurs pour les paramètres d'entrée selon une probabilité spécifiée par leurs FDP respectives. Les résultats de la simulation se présentent sous la forme de distributions des valeurs générées pour la donnée d'intérêt (ex. : C_{max}, SSC ou MET) (Figure 1.6). Pour tenter d'améliorer les analyses, il est possible dans certains cas de recourir à des approches bayésiennes de simulations de Monte-Carlo par chaînes de Markov (MCMC). Celles-ci permettent en effet de combiner l'information sur la variabilité issue de nouvelles données expérimentales d'une part, à l'information sur la variabilité contenue dans des distributions de paramètres définies *a priori* d'autre part, pour ultimement générer des distributions de paramètres *a posteriori*. Ces nouvelles distributions peuvent alors servir aux fins des simulations de Monte-Carlo classiques (ex. : Bois, 1999; 2001; Jonsson et Johanson, 2002).

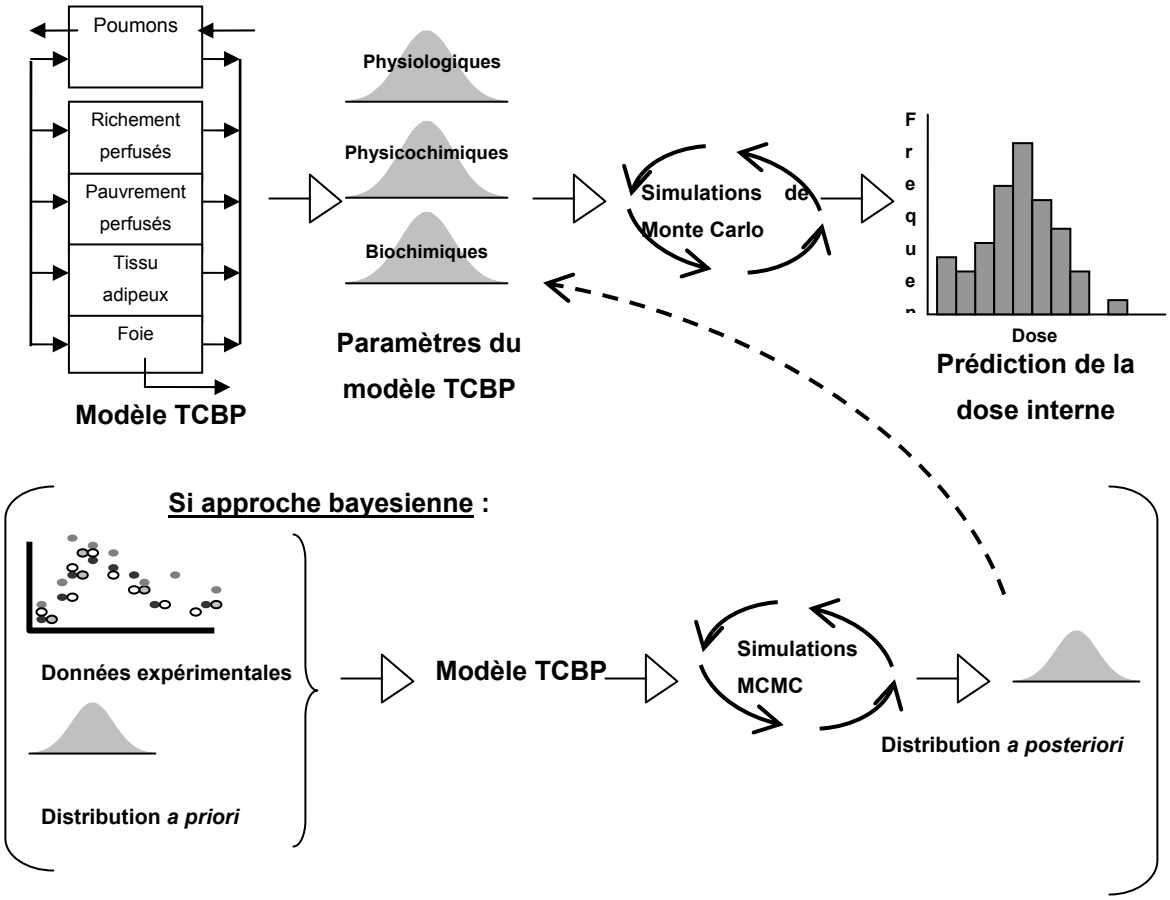


Figure 1.6 : Schéma illustrant le principe de la modélisation TCBP couplée à la méthode stochastique de simulation de Monte-Carlo, incluant l'approche bayésienne

De telles approches de simulation permettent donc de simuler la variabilité de la dose interne entre des individus types ou au sein de populations définies, suite à l'exposition à des xénobiotiques (Clewell et Andersen, 1996; Lipscomb *et al.*, 2004; Krishnan et Andersen, 2008; Gentry et Clewell, 2007). Comme on peut le voir aux Tableaux 1-V et 1-VI, ces approches ont été utilisées par de nombreux auteurs, mais ont surtout porté sur des populations adultes, quoique les enfants et les aînés aient été étudiés dans certains cas qui excluaient toutefois les approches bayésiennes (Tableau 1-V).

À partir des résultats obtenus dans de telles études pour divers sous-groupes de la population, il est possible de déterminer des rapports de doses internes enfants/adultes pour les études déterministes, et des FACHs suivant l'approche de l'OMS (IPCS, 2005) pour les études probabilistes. En effet, il ne suffit alors que de se référer aux valeurs de doses internes correspondant aux centiles requis (ex. : médiane, 5^{e}, 95^{e}). À noter que ce type d'approche de modélisation a également été abondamment utilisée en ce qui concerne les substances pharmaceutiques, mais par souci de concision, elles ne seront pas abordées au sein de cette thèse. Les sections suivantes consistent donc en une revue concernant les études de modélisation ayant généré des résultats permettant d'estimer la variabilité de la toxicocinétique des polluants lorsque non seulement les adultes sont considérés, mais également d'autres sous-groupes présumées plus sensibles que ceux-ci (Tableau 1-V). Certaines de ces études ont mené à des propositions de FACH. Par souci de synthèse, les études s'étant concentrées exclusivement sur les populations adultes ne seront pas décrites en détails, mais elles sont quand même énumérées dans le Tableau 1-VI. Cela inclut notamment des études sur l'impact du polymorphisme génétique sur la variabilité des doses internes (El-Masri *et al.*, 1999; Gentry *et al.*, 2002; Timchalk *et al.*, 2002). Cette dernière question a également été touchée par approche bayésienne (Jonsson et Johanson, 2001), mais toujours par souci de concision, de telles études sont aussi indiquées dans le Tableau 1-VI sans être approfondies. En effet, elles n'ont concerné la variabilité toxicocinétique que dans des populations adultes.

Tableau 1-V : Études sur la modélisation de la variabilité toxicocinétique entre les adultes et d'autres sous-groupes

Type de modélisation	Approche suivie	Population étudiée	Substance	Rapport le plus élevé obtenu[a)]	Référence
Déterministe	TCBP	adultes, enfants, aînés	trichloroéthylène, chloroforme, bromodichlorométhane, dibromochlorométhane, bromoforme	2,0	Haddad *et al.*, 2006
		adultes, nouveau-nés	caféine, théophylline	5,6; 7,7[b)]	Ginsberg *et al.*, 2004b
		adultes, nouveau-nés, enfants, aînés	isopropanol, nicotine, chlorure de vinyle, dichlorométhane, tétrachloroéthylène, dioxine	3,4; 3,9[b)]	Clewell *et al.*, 2004
		adultes, enfants	furanne	1,5	Price *et al.*, 2003a
		adultes, nouveau-nés, enfants, aînés	ozone, isopropanol, styrène, chlorure de vinyle, tétrachloroethylène	1,8; 2,0; 2,5; 11;4[b)]	Sarangapani *et al.*, 2003
		fœtus, nouveau-nés, femmes enceintes	isopropanol, nicotine, chlorure de vinyle, dichlorométhane, tétrachloroéthylène, dioxine	3	Gentry *et al.*, 2003
		adultes, enfants	dichlorométhane, tétrachloroéthylène, toluène, xylène, styrène, tétrachlorure de carbone, chloroforme, trichloroéthylène	2;2	Pelekis *et al.*, 2001
	Équilibre	adultes, enfants	gaz de catégorie 3	1,5; 2,3[b)]	Haber *et al.*, 2008
		adultes, nouveau-nés	Substrats gazeux des CYP2E1 et 1A2	0,2–2,7[b)]	Ginsberg *et al.*, 2005
		adultes, nouveau-nés	plomb	6,3	Beck *et al.*, 2002[c)]
Probabiliste	TCBP	adultes, enfants, nouveau-nés	acétone	2,5	Mörk et Johanson, 2010
		adultes, enfants	acrylamide	4,9	Walker *et al.*, 2007
		adultes, enfants,	chloroforme, bromodichlorométhane, dibromochlorométhane, bromoforme	3,9; 13,1[b)]	Tan *et al.*, 2007
			trichloroéthylène	6,5	Liao *et al.*, 2007a

adultes, nouveau-nés, enfants,	toluène	3,9	Nong *et al.*, 2006
adultes, nouveau-nés, enfants, aînés	dichlorométhane	2,3	Pelekis *et al.*, 2003
femmes enceintes, fœtus	méthylmercure	1,5	Clewell *et al.*, 1999
Équilibre femmes enceintes, fœtus	méthylmercure	1,5; 2,2[b)]	Swartout et Rice, 2000[c)], Stern, 1997[c)]

a) : Rapports des mesures moyennes de doses internes, ou FACHs correspondants, les plus élevés parmi les divers sous-groupes étudiées, arrondis à deux chiffres significatifs.

b) : Dépendamment de la substance et/ou de la mesure de dose interne considérée.

c) : Modèle non physiologique.

Tableau 1-VI : Exemples d'études de modélisation de la variabilité toxicocinétique des polluants environnementaux pour des populations adultes

Type de modélisation	Approche suivie	Substance	Référence
Probabiliste classique	TCBP	1,3-butadiène, dioxine	Beaudouin *et al.*, 2010
		acrylonitrile	Sweeney *et al.*, 2003
		trichloroéthylène	Lipscomb *et al.*, 2003
		chlorpyrifos	Timchalk *et al.*, 2002
		parathion, warfarine	Gentry *et al.*, 2002
		toluène	Tardif *et al.*, 2002
		chloroforme, tétrachlorure de carbone	Delic *et al.*, 2000
		dichlorométhane	El-Masri *et al.*, 1999
		trichloroéthylène	Simon, 1997
		benzène, chloroforme, tétrachlorure de carbone, dichlorométhane, méthylchloroforme, trichloroéthylène	Thomas *et al.*, 1996
		trichloroéthylène	Cronin *et al.*, 1995
	Équilibre	benzène, tétrachlorure de carbone, chloroforme, méthyle-chloroforme	Nong et Krishnan, 2007
		chloroforme	Yang *et al.*, 2010
Probabiliste bayésienne	TCBP	trichloroéthylène	Chiu *et al.*, 2009
		chloroforme	Liao *et al.*, 2007b
		tétrachloroéthylène	Covington *et al.*, 2007
		trichloroéthylène	Hack *et al.*, 2006
		benzène	Yokley *et al.*, 2006
		styrène	Jonsson et Johanson, 2002
		dichlorométhane	Jonsson et Johanson, 2001
		trichloroéthylène	Bois, 2000
		benzène	Bois *et al.*, 1996

1.2.2.4 Modélisations déterministes

1.2.2.4.1 Modèles TCBP

Parmi les études TCBP relatives aux différences entre les adultes et les enfants, la première est celle de Pelekis *et al.* (2001) qui ont simulé les concentrations sanguines et tissulaires en substances mères chez les adultes et les enfants âgés de un à deux ans suite à l'exposition par inhalation à divers composés organiques volatils (COV). Chez l'adulte, des simulations ont été effectuées en considérant des valeurs dites « élevées » ou « basses » pour les paramètres d'entrée du modèle, afin d'estimer la variabilité plausible dans les concentrations sanguines adultes. Les simulations ont été répétées en considérant des valeurs dites « moyennes » pour l'enfant. Les résultats montrent que les rapports entre les concentrations obtenues avec les paramètres élevés ou bas chez l'adulte variaient entre 0,17 (styrène dans les tissus adipeux) et 2,85 (tétrachlorure de carbone dans les tissus richement perfusés), dépendamment des substances (toluène, xylène, styrène, dichlorométhane, tétrachloroéthylène, chloroforme, trichloroéthylène, tétrachlorure de carbone) et du milieu considéré (sang artériel ou veineux, dans les tissus fortement ou faiblement perfusés, les tissus adipeux ou le foie). Les rapports basés sur la comparaison des résultats obtenus pour l'adulte selon les paramètres générant une clairance élevée avec ceux de l'enfant moyen variaient entre 0,033 (styrène dans le foie) et 2,2 (tétrachlorure de carbone dans le sang artériel). Tous ces résultats étaient répliqués similairement par simulation à l'état d'équilibre, en tenant compte des coefficients de partage tissu:sang. Ces résultats peuvent être considérés comme les premières tentatives de calculs de FACH à partir de données modélisées, quoique non probabilistes.

Price *et al.* (2003a) ont par la suite modélisé les différences de concentrations sanguines à l'équilibre de furanne entre adultes et enfants de 6, 10 et 14 ans suite à une inhalation de 1 µg/L pendant 30 heures. Le métabolisme était assumé comme débit-dépendant et le rapport des concentrations sanguines enfant/adulte le plus élevé était de 1,5, observé avec

l'enfant de 6 ans. Également, Ginsberg *et al.* (2004b) ont décrit le développement d'un modèle TCBP pour comparer le devenir de la caféine et de la théophylline dans l'organisme d'enfants nouveau-nés (1-7 jours) et de l'adulte. Leurs résultats modélisés montrent une demi-vie dans le sang plus courte chez l'adulte par un facteur de 7,7 et 5,6 pour la caféine et la théophylline, respectivement.

Gentry *et al.* (2003) ont effectué une modélisation TCBP de l'exposition pré et post-natale via l'ingestion d'eau potable pour six substances aux propriétés physico-chimiques distinctes, soit l'isopropanol, le chlorure de vinyle, le dichlorométhane, le tétrachloroéthylène, la dioxine et la nicotine. Leurs résultats font ressortir des différences de concentrations sanguines entre la mère d'une part, et l'enfant âgé de 1, 3 ou 6 mois, ainsi que le fœtus, d'autre part, qui étaient toujours en deçà d'un facteur de 3, peu importe l'entité toxique considérée. Il en va de même lorsque la comparaison était faite entre les individus type de divers groupes d'âge (moins de 6 mois, 6 mois à 5 ans, 5 à 25 ans) et l'adulte de 25 ans, sauf dans le cas de la nicotine (Clewell *et al.*, 2004). Dans ce dernier cas, un rapport de 3,4 a été obtenu en considérant les moins de 6 mois. Le rapport pouvait varier lorsque les voies d'exposition étaient distinguées. Ainsi, pour l'isopropanol, le rapport était de 2,0, 1,9 et 1,1 pour l'exposition par inhalation, par ingestion et par voie cutanée respectivement, alors que pour son métabolite, l'acétone, ces rapports étaient de 3,9, 1,1 et 2,2, respectivement. Ces résultats ont donc fait ressortir l'importance de la voie d'exposition sur l'ampleur de la variabilité interindividuelle des doses internes, ce qui laisse entrevoir un impact potentiellement important sur la valeur du FACH.

La modélisation TCBP a été utilisée par Sarangapani *et al.* (2003) pour illustrer les différences entre tous les groupes d'âge ainsi que selon le sexe dans la toxicocinétique de quatre gaz à action systémique, soit l'isopropanol, le styrène, le chlorure de vinyle et le tétrachloroéthylène. Outre les variations physiologiques (débits sanguins, volumes

tissulaires, etc.), les variations dans les activités des enzymes hépatiques impliquées (ADH, CYP2E1) et dans la filtration glomérulaire étaient considérées. Pour le tétrachloroéthylène, très peu de variations étaient observées sur la base de la concentration sanguine en substance mère. Par contre, les rapports des concentrations sanguines entre les divers groupes et l'adulte de 25 ans variaient de manière importante pour le métabolite circulant, soit entre 0,21 pour les garçons de 1 mois et 1,41 pour les femmes de 75 ans. Par contre, la production de métabolites suivait les variations relatives à l'activité enzymatique du foie. Pour l'isopropanol, le rapport variait entre 0,94 pour l'homme de 50 ans et 1,78 pour la fille de 3 mois, pour la substance mère. Le rapport variait entre 0,8 pour l'homme de 50 ans et 11,44 pour la fille de 1 mois, lorsque la concentration de métabolite circulant (acétone) était considérée. Le rapport était supérieur à la valeur par défaut de 3,16 jusqu'à l'âge de 1 an. Ensuite, la concentration sanguine d'acétone diminuait jusqu'à l'âge adulte, suivant le développement des CYP2E1 qui le métabolisent. Les rapports obtenus pour le styrène variaient entre 0,81 et 2,5 selon l'âge, le sexe et l'entité toxique (substance mère ou métabolite réactif), et entre 0,82 et 1,95 pour le chlorure de vinyle.

Mielke *et al.* (2005) et Abraham et coll. (2005a, 2005b) ont eu recours à la modélisation TCBP pour étudier l'évolution du rapport des concentrations sanguines entre l'adulte moyen et des enfants d'âge variable (de nouveau-né à 15 ans) en fonction de la durée et du niveau d'exposition. Ainsi, Mielke *et al.* (2005) ont obtenu un rapport maximal de près de 1,9, considérant les nouveau-nés, lors de l'exposition à 1 ppm de dichlorométhane durant 8 heures. À 10 000 ppm, ce rapport maximal baissait à environ 1,4 au début de l'exposition, et jusqu'à 1,15 au bout de 3 heures. Pour une exposition de 8 heures, ce rapport était d'environ 1,9 tant que la concentration était inférieure à 100 ppm, mais baissait rapidement jusqu'à 1,15 pour des expositions plus élevées. Les auteurs expliquaient ces résultats par l'effet de la saturation du métabolisme qui apparaissait plus tôt chez les enfants que chez les adultes, à faibles concentrations ou quand l'exposition était de courte durée. Le même effet était observé pour le styrène (Abraham *et al.*, 2005a), mais les rapports de concentrations sanguines étaient plus élevés, présumément en raison d'un coefficient de partage sang:air

(Pb) plus élevé pour le styrène (Abraham *et al.*, 2005b). En effet, plus le Pb est élevé, moins la clairance pulmonaire, pour laquelle les enfants sont avantagés par rapport à l'adulte, est importante dans la clairance systémique totale. Ainsi, ce rapport atteignait 3,9 à faible concentration (100 ppm) mais baissait à 2,5 si la concentration dans l'air quadruplait, pendant 8 heures. Également, ce rapport dépassait légèrement 2 au début de l'exposition à forte concentration (1000 ppm) avant de baisser à environ 1,6 au bout de 8 heures.

Un modèle TCBP multivoies a été utilisé par Haddad *et al.* (2006) pour étudier les différences de doses totales reçues (C_{max}, SSC et quantité métabolisée) en fonction de l'âge suite à une exposition à des contaminants de l'eau potable (trichloroéthylène, trihalométhanes) durant la prise de bains et de douches. Les simulations ont été effectuées en considérant des paramètres d'entrée du modèle avec des valeurs moyennes, extrêmes pour la taille et le poids (5^e et 95^e centile), ou extrêmes pour la taille, le poids et les coefficients de partage tissu:air et sang:air. Les résultats montrent des différences pouvant atteindre un facteur de 2 dans la dose interne entre l'adulte et l'enfant, dépendamment de l'âge de ce dernier, alors que les différences n'étaient pas significatives avec les aînés.

1.2.2.4.2 Algorithmes à l'équilibre

En utilisant un algorithme à l'équilibre, Haber *et al.* (2008) ont simulé les différences de concentrations sanguines de produit mère et de métabolite stable ainsi que de production de métabolites réactifs entre l'adulte et des enfants de 3 mois, 1, 5 et 10 ans suite à une exposition par inhalation à une concentration constante de divers gaz de catégorie 3 de la U.S. EPA (c.-à-d. non réactifs, à action systémique). Ceux-ci présentaient un coefficient de partage sang:air variant entre 1 et 50, étaient éliminés par clairance hépatique et pulmonaire, étaient peu hydrosolubles et causaient leur effet toxique par action systémique (ex. : styrène). Divers scénarios impliquant de faire varier le ratio d'extraction hépatique

entre 0 et 1 et considérant des clairances intrinsèques entre 0,1 L/h ("faible") ou 1000 L/h ("élevée"), de même que la prise en compte des variations dépendante de l'âge dans le contenu hépatique des enzymes impliquées dans la biotransformation (CYP2E1, ADH), ont été envisagés.

Les différences obtenues des concentrations sanguines modélisées du produit mère suggèrent un rapport maximal des concentrations sanguines d'environ 2,3, observé entre les adultes et les enfants de 3 mois, pour les substances à clairance intrinsèque « élevée », soit 1000 L/h. En ce qui concerne la production de métabolites réactifs, les résultats obtenus ont suggéré un rapport maximal de 1,5 (pour les enfants de 10 ans) lorsque les substances présentaient un métabolisme débit-dépendant, c.-à-d. à clairance intrinsèque « élevée ». Les variations dans la filtration glomérulaire tout comme dans le contenu en enzymes hépatiques étaient déterminantes sur la concentration sanguine d'un métabolite stable, pour lequel le rapport des concentrations sanguines enfant/adulte était généralement inférieur à 1. Dans tous les cas, le rapport des concentrations sanguines entre l'enfant et l'adulte augmentait en fonction du coefficient de partage sang:air, suivant la logique décrite plus haut pour les études de Mielke *et coll*. (2005) et Abraham *et coll*. (2005a).

Ginsberg *et al.* (2005) ont utilisé une équation du calcul à l'équilibre de la concentration sanguine artérielle et de la dose de métabolites au foie durant l'inhalation et ont calculé des rapports de ces doses internes entre l'enfant de 3 mois et l'adulte pour des gaz théoriques de catégorie 3 et métabolisés par le CYP2E1 et le CYP1A2. Ainsi, ces substrats théoriques présentaient des coefficients de partage sang:air (Pb) de 1, 10 ou 50 et des rapports clairance/débit hépatique variant entre 0,3 et 100. Pour les substrats du CYP2E1, les rapports des concentrations sanguines variaient respectivement entre 1,1 et 1,7 pour Pb = 50, entre 1,1 et 1,3 pour Pb = 10, mais demeuraient stables à environ 1 pour Pb = 1. Les valeurs les plus élevées étaient obtenues pour le rapport clairance/débit hépatique de 0,3. En ce qui concerne la dose de métabolites au foie, les rapports croissaient de manière

constante avec le rapport clairance/débit hépatique, pour chaque valeur de Pb, et variaient entre 1,0 et 1,9. Les mêmes tendances étaient obtenues pour les substrats théoriques du CYP1A2, mais avec des rapports plus élevés de concentration sanguine, c.-à-d. pouvant atteindre 1,5 lorsque Pb = 10 ou 2,7 lorsque Pb = 50.

Beck *et al.* (2002) ont utilisé deux modèles cinétiques humains du plomb ayant recours à un « facteur de pente biocinétique » pour les enfants de 2 ans et pour les adultes afin de déterminer les apports requis pour atteindre une plombémie sanguine de référence. Celle-ci était établie à 11,1 µg/dl chez la femme enceinte (pour protéger le fœtus) et 10 µg/dl chez l'enfant. Sur la base de leur modélisation, les apports devraient être identiques chez les adultes et les enfants. Toutefois, ces résultats se sont basés sur des biodisponibilités gastro-intestinales différentes entre la femme enceinte (8 %) et l'enfant (50 %). Avec des valeurs de biodisponibilité identiques, l'apport requis par l'enfant pour atteindre la plombémie de référence aurait été inférieur à l'apport chez l'adulte par un facteur de 6,25 (50/8), ce qui justifierait l'application d'un FACH équivalent.

1.2.2.5 Modélisations probabilistes

1.2.2.5.1 Modèles TCBP

À l'aide de simulations de Monte-Carlo, Clewell *et al.* (1999) ont introduit la variabilité dans les paramètres décrivant un modèle TCBP à 17 compartiments maternels et quatre compartiments fœtaux. Ils ont ainsi généré des distributions de doses d'ingestion de méthylmercure requises pour atteindre une concentration fixe dans les cheveux chez les femmes américaines en âge de procréer. Les résultats obtenus sous-tendent un rapport [médiane/5^{e} centile] de 1,5, peu importe la concentration fixe visée.

Une étude de modélisation TCBP « vie-durant » effectuée par Pelekis *et al.* (2003) sur l'inhalation d'une concentration constante de dichlorométhane a permis de constater que la variabilité interindividuelle pour un même âge était assez constante. Ainsi, le facteur de variabilité pour les individus d'un même âge, c.à.d. le rapport [95^{e} centile/médiane] de la concentration sanguine, variait entre 1,9 et 2, dépendant du tissu et de l'âge considéré. Toutefois, lorsque la comparaison se faisait entre des enfants de 1 à 5 ans (au numérateur) et l'adulte (au dénominateur), le rapport obtenu était d'environ 2,3.

Nong *et al.* (2006) ont utilisé un modèle TCBP pour simuler la variabilité inter et intragroupe dans la cinétique du toluène, sur la base de la SSC pour le sang veineux. Ils ont notamment considéré la variabilité associée au contenu en enzymes CYP2E1 dans les divers sous-groupes de la population classée par âges (< 1 mois, métaboliseurs lents et rapides, 1 mois–1 an, 1–11 ans, 12-17 ans, adultes). L'inhalation de 1 ppm sur 24 heures était modélisée et les résultats obtenus suggèrent des valeurs de facteur d'ajustement pour la cinétique humaine variant entre 1,35 (pour les 12–17) et 3,88 (pour les moins de 1 mois et métaboliseurs lents), par rapport à l'adulte. À l'intérieur de chaque groupe, le facteur de variabilité oscillait entre 1,07 (nouveau-nés, métaboliseurs lents) et 1,48 (adultes).

Dans une autre étude où le contenu en enzymes hépatiques a été pris en considération, Walker *et al.* (2007) ont simulé la variabilité dans la dose interne d'acrylamide suite à une exposition par ingestion bolus de 1 µg/kg chez des enfants de 0 à 10 ans regroupés en cinq groupes d'âges (dont quatre avant 1 an) ainsi que chez l'adulte. Les paramètres physiologiques d'entrée étaient fixes pour chaque groupe, mais le contenu en enzymes de biotransformation impliquées (CYP2E1, glutathion s-transférase, époxyde hydrolase) était exprimé sous forme de distributions de valeurs. Les résultats basés sur la SSC suggèrent un FACH de 4,9 si on considère le rapport entre le 99^{e} centile chez les nouveau-nés et la médiane chez l'adulte.

La variabilité dans l'exposition multivoies au trichloroéthylène et aux trihalométhanes a été étudiée respectivement par Tan *et al.* (2007) et Liao *et al.* (2007a). Ces auteurs ont effectué la modélisation TCBP probabiliste des concentrations sanguines retrouvées suite à l'exposition à l'eau potable lors de l'ingestion et de la prise de douche. La modélisation considérait un seul groupe populationnel comprenant les individus de plus de 12 ans aux États-Unis. Les rapports [95^{e} centile/médiane] des concentrations sanguines pour le chloroforme, le bromodichlorométhane, le dibromochlorométhane et le bromoforme étaient respectivement de 5,6, 3,9, 5,8 et 13,1. Les concentrations modélisées étaient similaires aux mesures biologiques effectuées dans le cadre de l'enquête NHANES (Tan *et al.*, 2007). Dans le cas du trichloroéthylène, le rapport observé était de 16,2. Cependant, dans ce dernier cas, les auteurs font état d'une surestimation des prédictions du modèle pour les valeurs supérieures au 90^{e} centile en comparaison des valeurs de NHANES. Cette surestimation serait de l'ordre d'un facteur de 2,5. On peut en déduire que le rapport réel pour les concentrations sanguines en trichloroéthylène serait plus de l'ordre de 6,5 (16,2/2,5).

Finalement, Mörk et Johanson (2010) ont récemment évalué la distribution populationnelle de la concentration sanguine à l'état stationnaire d'acétone, en considérant ou non la production endogène, pour une inhalation continue à la RfC de 29 ppm. Ce faisant, les auteurs ont considéré les proportions démographiques suédoises de chaque groupe d'âge considéré (adultes, bébés de 3 mois, enfants de 1, 5 et 10 ans, adolescents de 15 ans). Les adultes et les adolescents étaient aussi répartis selon le sexe. Considérant la population entière, des FACHs de 1,9 et 2,1 ont été obtenus sur la base respectivement du 95^{e} et du 97,5^{e} centile des concentrations sanguines d'acétone, par rapport à la médiane, et excluant la production endogène. En considérant les sous-groupes pris séparément, les valeurs correspondantes les plus élevées étaient de 2,4 et 2,5 obtenues pour des enfants de 5 ans, et n'étaient jamais inférieures à 2,2 (obtenues pour les bébés).

1.2.2.5.2 Algorithmes à l'équilibre

Stern *et al.* (2002) ont proposé des valeurs de FACH pour le méthylmercure sur la base de la variabilité des résultats de modélisations cinétiques obtenus par deux modèles à un compartiment illustrant la relation entre l'ingestion de méthylmercure et la concentration dans les cheveux ou le sang de femmes enceintes (Stern, 1997; Swartout et Rice, 2000). Dans les deux cas, des simulations de Monte-Carlo ont été effectuées afin de simuler la variabilité dans les paramètres physiologiques affectant la cinétique du méthylmercure dans l'organisme de la femme enceinte. Ainsi, le rapport entre la médiane et le cinquième centile de la distribution de l'ingestion de méthylmercure requise pour atteindre une concentration fixe dans les cheveux (11 µg/g) variait selon le modèle utilisé entre 1,5 et 2,2. Si une concentration fixe dans le sang était considérée comme point de référence, ces rapports variaient entre 1,4 et 2,1. Sur la base de ces résultats, et puisqu'ils ont été obtenus pour un sous-groupe sensible (femme enceinte), il est suggéré par les auteurs qu'une valeur de 3 attribuée à un FACH pour le méthylmercure devrait être suffisante. Ils émettent toutefois la réserve que ces résultats découlent de concentrations pour les cheveux et le sang, alors que l'organe cible du méthylmercure est le cerveau. Une variabilité supplémentaire pourrait donc découler du transfert du méthylmercure du sang vers le cerveau.

1.2.2.6 Bilan

De manière générale, on constate donc que la variabilité interindividuelle des doses internes a été étudiée en considérant principalement des adultes et des enfants, mais d'autres sous-groupes ont aussi été considérés en quelques occasions. En particulier, les femmes enceintes ont été considérées lors d'études portant sur deux contaminants particulièrement pertinents en regard de leur état physiologique, soit le plomb (Beck *et al.*, 2002) et le méthylmercure (Clewell et al., 1999; Swartout et Rice, 2000; Stern, 1997), ainsi que sur des COVs (Gentry *et al.*, 2003). Les aînés ont aussi fait l'objet d'analyses en regard de leur

exposition aux COVs (Sarangapani *et al.*, 2003; Pelekis *et al.*, 2003). Mais il demeure que ce sont les enfants qui ont été les plus étudiés et ce, en considérant des âges très variés (de quelques jours à 15 ans). Toutefois, il ressort des résultats revus ici que les différences de doses internes suffisamment importantes par rapport à l'adulte pour suggérer un FACH supérieur à la valeur par défaut de 3,16 ont été obtenues seulement pour les tout jeunes enfants, généralement âgés de 3 mois ou moins. C'était particulièrement le cas pour la théophylline (7,7) et la caféine (5,6), deux substrats du CYP1A2 (Ginsberg *et al.*, 2004b); le plomb (6,3, Beck *et al.*, 2003); l'acrylamide (4,9, Walker *et al.*, 2007), ainsi que l'acétone, le métabolite de l'isopropanol (3,9 et 11,4, obtenus respectivement par Clewell *et al.*, 2004 et Sarangapani *et al.*, 2003). En ce qui concerne les autres COVs, seuls Nong *et al.* (2006) ont obtenu un facteur supérieur à 3,2, soit 3,9 pour le toluène inhalé. Toutefois, en considérant des enfants de 12 ans, Tan *et al.* (2007) ainsi que Liao *et al.* (2007a) ont obtenu des facteurs atteignant 13,1 et 6,5 pour les trihalométhanes et le trichloroéthylène respectivement, mais en considérant l'exposition multivoies aux contaminants de l'eau potable par l'inhalation et le contact cutané lors de la prise de bains et de douches, ainsi que l'ingestion. À noter qu'à l'exception de l'acétone, toutes ces valeurs ont été obtenues lorsque la substance mère était considérée pour l'analyse, et non pas les métabolites.

1.3 Problématique

La détermination de FACH sur la base de distributions populationnelles de doses internes ou de paramètres y étant reliés (demi-vies, clairances, etc.) pour des substances spécifiques est une approche intéressante du point de vue scientifique, mais demeure peu appliquée par les divers organismes établissant les valeurs de référence. Ainsi, l'IRIS n'a à ce jour suivi ce processus que pour cinq substances (DeWoskin *et al.*, 2007). De plus, l'application de ce processus a parfois mené à des résultats étranges. Ainsi, en appliquant la directive de l'IPCS, Palmer et Brent (2005) ont proposé pour l'éthylène glycol un FI de 10,24, basé sur les valeurs par défaut arrondies pour les composantes toxicocinétique et toxicodynamique.

De plus, un facteur de 10 découlant de la multiplication des valeurs arrondies à un seul chiffre significatif de ces facteurs (3) a été retenu pour le 1,3-butadiène par Grant *et al.* (2010) même si dans les faits, ce calcul donne une valeur de 9… Pour diverses raisons, la communauté scientifique est ainsi partagée entre les tenants du maintien de l'approche par défaut et ceux de l'approche de détermination des FASCs basée sur les données (SA, 2002). Cette dichotomie transpire même du document officiel de l'IPCS, comme le témoigne le passage suivant sur la détermination d'un FASC combiné de 9, sensé remplacer la valeur par défaut de 100 pour une substance théorique (IPCS, 2005):

> *« The total factor of 9 means that a different toxic end-point, observed in animal studies at higher doses but with default 100-fold factor, may become the critical effect. »*

À la lumière de la revue effectuée ici, il appert que dans le cas du FACH, les réticences quant à sa détermination puissent découler du manque de données requises à cet égard d'une part, et possiblement des incertitudes quant aux conséquences qui en résulteraient du point de vue de gestion des risques d'autre part. En fin de compte, la valeur par défaut (3,16) est donc encore à ce jour très généralement utilisée et appliquée indépendamment :

- de la voie d'exposition considérée (ex. : RfD *vs* RfC);
- de la durée et de l'intensité de l'exposition (ex. : aigü *vs* chronique);
- des propriétés physico/biochimiques et de l'entité responsable de l'effet toxique (substance mère ou métabolites) des substances;
- des sous-groupes présumés sensibles qu'on cherche à protéger;
- des caractéristiques de la population générale.

La section 1.2.2.2 a mis en évidence que les données expérimentales permettant d'évaluer, au moins partiellement, la justesse de la valeur de 3,16 en regard des cinq points ci-haut concernent principalement les substances pharmaceutiques. Or, ces substances diffèrent sensiblement des contaminants environnementaux. Ainsi, elles n'empruntent pas nécessairement les mêmes voies d'entrée et métaboliques et présentent une distribution souvent fort différente de plusieurs polluants lipophiles, puisque les médicaments sont généralement plus hydrosolubles. De plus, les données expérimentales sur les substances pharmaceutiques n'ont pas été colligées dans des conditions d'exposition chronique à faible dose, plus pertinentes à l'étude des contaminants environnementaux. Il est donc hasardeux d'extrapoler à ces derniers les conclusions pouvant être tirées de données expérimentales concernant strictement les médicaments.

Pour contourner cette difficulté, la modélisation TCBP a été utilisée à quelques occasions dans la littérature, tel que décrit dans la section 1.3.3. Toutefois, à la lumière de cette revue, des lacunes persistent et plusieurs questions demeurent sans réponse. Ainsi, on constate que la majorité des études probabilistes ayant été réalisées se sont limitées à simuler la variabilité à l'intérieur d'un seul sous-groupe de la population, la plupart du temps les adultes (voir Tableaux 1-V et 1-VI). Si cela peut s'avérer intéressant du point de vue des expositions en milieu de travail, l'application des informations découlant de telles études au cas de la population générale, incluant les non-adultes, exposées aux contaminants de l'environnement global, est limitée. Les études comparatives entre l'adulte et les autres sous-groupes de la population demeurent relativement peu fréquentes. Quand cela s'est fait, l'approche déterministe était suivie dans la majorité des cas. Cela n'a pas permis de caractériser la variabilité à l'intérieur de chaque sous-groupe. Par ailleurs, les rares analyses « adultes *vs* enfants » probabilistes publiées portaient presque exclusivement sur des expositions par inhalation, généralement à concentration et durée fixe. Ainsi, on ne connaît pas bien comment les **conditions d'exposition, incluant la voie, la durée et l'intensité,** affectent la valeur du FACH pour des polluants, malgré que les résultats de Clewell *et al.*

(2004), Mielke *et al.* (2005) et Abraham *et al.* (2005a, b) suggèrent un effet en ce sens. Également, on n'a pu à ce jour caractériser de réelles tendances concernant la valeur du FACH **en fonction des caractéristiques physico-biochimiques des contaminants**. Pourtant, les résultats de Dorne *et al.* (2005) et Ginsberg *et coll*. (2005), notamment, suggèrent qu'une telle dépendance existe. La considération des différentes entités toxiques (substances mères ou métabolites) n'a jamais été abordée non plus. Finalement, l'approche de l'IPCS (2005) suppose qu'une fraction de la population ne sera pas couverte par le FACH, ce qui est une question importante, comme en fait foi l'intérêt soulevé par cette question dans des études passées (Calabrese, 1985; Hattis *et al.*, 1987; 1999b; Renwick et Lazarus, 1998). De plus, la littérature consultée ne permet pas de déterminer clairement si le « référent » utilisé, soit l'individu dont la valeur du paramètre pharmacocinétique d'intérêt sera utilisé au dénominateur de la formule utilisée pour calculer le FACH, consiste en un adulte médian ou en un individu médian d'une population incluant adultes et non-adultes, **et si ce choix peut faire varier la fraction de la population qui est couverte par le FACH** (Mörk et Johanson, 2010). La présente thèse vise ainsi à réponde aux nombreuses interrogations soulevées ci-haut.

1.4 Hypothèse générale et objectifs

L'hypothèse de départ est que **la variabilité toxicocinétique interindividuelle, et en corollaire le FACH, dépend 1) des conditions d'exposition, 2) des caractéristiques physico/biochimiques des substances, 3) de l'entité toxique d'intérêt et 4) des sous-groupes de la population, populations générales et « référents » considérés**.

L'objectif de cette étude consiste donc à **caractériser le FACH sur la base des diverses entités toxiques (substances-mère et métabolite) et considérant les divers sous-groupes de la population en fonction des conditions d'exposition (voie, durée et intensité), des propriétés physico-biochimiques des substances et des caractéristiques de la population générale et du référent considéré.**

1.5 Organisation de la thèse

La présente thèse est organisée selon le format par articles. Les articles suivent l'ordre déterminé par la poursuite de l'objectif décrit ci-haut. Dans tous les cas, le principe général de détermination des FACHs selon l'approche de l'IPCS (2005) a été suivi, pour chaque scénario d'exposition et sous-groupe de la population ou population générale modélisée, de même que pour toutes les entités toxiques considérées (substances mères ou métabolites). Ainsi, **le premier article** visait à déterminer l'impact de la voie d'exposition sur le FACH. La modélisation TCBP probabiliste a été utilisée pour simuler la variabilité des doses internes de quatre COVs selon trois scénarios d'exposition, soit par la voie orale (ajustée au poids corporel), par inhalation et par voie cutanée. Ces scénarios ont été simulés pour des adultes, des femmes enceintes, des ainés, des nouveau-nés et des jeunes enfants. Le **second article** visait pour sa part à caractériser le FACH en fonction de la durée et de l'intensité de l'exposition. Un modèle TCBP probabiliste a été utilisé pour simuler la distribution des doses internes de quatre COVs chez des adultes, des jeunes enfants, des nouveau-nés et des femmes enceintes suivant des scénarios d'exposition par inhalation variables en durée et en intensité.

Dans le **troisième article**, l'impact des propriétés physico/biochimiques sur la valeur du FACH a été étudié à l'aide un algorithme toxicocinétique à l'état stationnaire permettant de calculer, dans divers sous-groupes (adultes, nouveau-nés, aînés, femmes enceintes), la concentration sanguine à l'équilibre en substance mère et le taux de production de métabolites lors d'expositions chroniques. Les expositions considérées consistaient en une exposition par inhalation à concentration fixe d'une part, et à une exposition systémique identique en mg/kg-d d'autre part. Pour des substrats théoriques de diverses voies métaboliques pertinentes aux contaminants environnementaux, soit le CYP2E1, le CYP1A2, le CYP3A4 et l'ADH, des matrices de FACH élaborée en fonction du Pb (variant entre 1 et 10 000) et du ratio d'extraction hépatique (E) chez l'adulte moyen (variant entre 0,01 et 0,99) ont été générées. Cela a permis d'obtenir et d'expliquer des tendances quant à

la valeur du FACH selon les caractéristiques physico-biochimiques que reflètent le Pb et le E, soit respectivement la solubilité sanguine et l'ampleur du métabolisme hépatique des substances.

Tandis que le troisième article a mis en lumière l'impact de la clairance systémique sur le FACH, **le quatrième article** a en plus pris en compte la clairance présystémique pour l'évaluation du FACH lors de l'exposition par voie orale. Dans cet article, l'algorithme toxicocinétique à l'état stationnaire utilisé dans l'article précédent a été modifié pour refléter le phénomène physiologique de premier passage hépatique pertinent pour la voie orale. Encore une fois, des matrices de FACH ont été générées pour des substrats théoriques, ce qui a permis, par comparaison avec l'article précédent, de clarifier l'impact du premier passage hépatique sur le FACH des contaminants ingérés.

Finalement, **le cinquième article** portait sur l'évaluation de l'impact du référent considéré et de la composition de la population sur la valeur du FACH. Ainsi, pour l'inhalation chronique de benzène ou de 1,4-dioxane, des distributions populationnelles théoriques de doses internes ont été « reconstruites » en fonction de la représentativité de chaque sous-groupe au sein de la population canadienne. L'impact d'une modification de la représentativité démographique des sous-groupes sur la distribution populationnelle des doses internes a été évalué. Le FACH a été calculé selon plusieurs approches, que ce soit en considérant la distribution des doses internes dans la population entière ou encore dans chaque sous-groupe, en faisant varier le référent considéré. De plus, l'étude a évalué le niveau de protection apporté par le FACH à chaque sous-groupe composant la population générale canadienne dans chaque cas.

Corps de la thèse : articles

2 Article I : *Evaluation of the impact of the exposure route on the Human Kinetic Adjustment Factor*

Valcke, M. et Krishnan, K.

Valcke, M., and Krishnan, K. (2011). Evaluation of the impact of the exposure route on the Human Kinetic Adjustment Factor. Regulatory Toxicology and Pharmacology 59, 258-269. doi:10.1016/j.yrtph.2010.10.008

EVALUATION OF THE IMPACT OF THE EXPOSURE ROUTE ON THE HUMAN KINETIC ADJUSTMENT FACTOR

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Abstract

The objective of this study was to assess the impact of the exposure route on the human kinetic adjustment factor (HKAF), for which a default value of 3.16 is used in non-cancer risk assessment. A multiroute PBPK model was modified from the literature and used for computing the internal dose metrics in adults, neonates, children, elderly and pregnant women following three route-specific scenarios to chloroform, bromoform, tri- or per-chloroethylene (TCE or PERC). These include 24-h inhalation exposure, body-weight adjusted oral exposure and 30 minute dermal exposure to contaminated drinking water. Distributions for body weight (BW), height (BH) and hepatic cytochrome P450 2E1 (CYP2E1) content were obtained from the literature whereas model parameters (flows, volumes) were calculated from BW and BH. Monte Carlo simulations were performed and the HKAF was calculated as the ratio of the 95th percentile value of internal dose metrics in subpopulation to the 50th percentile value in adults. On the basis of the area under the parent compound's arterial blood concentration *vs* time curve (AUC_{pc}), highest HKAFs were obtained in neonates for every scenario considered, and were the highest for bromoform (range: 3.6 - 7.4). Exceedance of the default value based on AUC_{pc} was also observed for an oral exposure to chloroform in neonates (4.9). In all other se, HKAFs remained below the default value. Overall, this study has pointed out the dependency of the HKAF on the exposure route, dose metrics and subpopulation considered, as well as characteristics of the chemicals investigated.

Keywords: Chemical-specific adjustment factors (CSAF), Drinking water contaminants, Exposure route, Human kinetic adjustment factor (HKAF), Physiologically-based pharmacokinetic (PBPK) modelling, VOCs.

LIST OF ABBREVIATIONS AND ACRONYMS

ACN	**acrylonitrile**
ADH	**alcohol dehydrogenase**
AMET	**amount metabolized**
AUC_{pc}	**area under blood concentration *vs* time curve of the parent compound**
AUC_{met}	**area under the blood concentration *vs* time curve of the circulating metabolite**
BH	**body height**
BSA	**body surface area**
BW	**body weight**
CSAF	**chemical-specific adjustment factor**
CYP2E1	**cytochrome P-450 2E1**
DW	**drinking water**
DWC	**drinking water contaminants**
E_{ren}	**renal extraction ratio**
Fracmet	**fraction metabolized**
GFR	**glomerular filtration rate**
HKAF	**human kinetic adjustment factor**
IP	**isopropanol**
Km	**Michaëlis-Menten constant**
K_u	**urinary excretion constant**
MC	**Monte Carlo**
MSP	**microsomal protein**
Pb	**blood:air partition coefficient**
PBPK	**physiologically-based pharmacokinetic**
PC	**partition coefficient**
PDF	**probability density function**
PERC	**perchloroethylene**
PW	**pregnant women**
Qc	**cardiac output**
Qk	**kidney blood flow**
Ql	**liver blood flow**
Qp	**alveolar ventilation rate**
RfC	**reference concentration**
RfD	**reference dose**
SD	**standard deviation**
SEM	**standard error of the mean**
TCA	**trichloroacetic acid**
TCE	**trichloroethylene**
UF	**uncertainty factor**
Vf	**volume of fat**
Vmax	**maximum rate of metabolism**
Vl	**volume of liver**
VOC	**volatile organic compound**
VT	**variability term**

2.1 Introduction

A default uncertainty factor (UF) of 10 is usually applied to account for the human interindividual variability when establishing the reference doses (RfD) and the reference concentrations (RfC) (Dourson *et al.*, 1996; Dourson and Stara, 1983; U.S. EPA, 2002). Thus, the application of this UF implies that for both ingested and inhaled toxicants, the internal dose and adverse response may vary by at least an order of magnitude within the human population, even though RfDs and RfCs are expressed in different units (mg/kg-d *vs* mg/m^3). It has been proposed that the interindividual variability in toxicokinetics as well as toxicodynamics of chemicals can be considered separately and that both components be attributed a default value of $\sqrt{10}$, or 3.16 (Dorne and Renwick, 2005; IPCS, 1994). The adequacy of this default value for specific chemicals can be assessed and replaced as appropriate by quantifying chemical-specific adjustment factors (CSAF) as proposed by the International Programme on Chemical Safety (IPCS, 2005; Meek *et al.*, 2002).

Under this approach, the CSAF for interindividual variability in toxicokinetics, or alternatively referred to as the human kinetic adjustment factor (HKAF), has been determined based on a probability density function (PDF) of pharmacokinetic parameters (e.g., half-life) in the studied population (IPCS, 2005; Meek *et al.*, 2002). For a unimodal PDF, the HKAF is determined as the ratio between an upper percentile value (e.g. 95^{th}) and the central tendency (e.g. median). If this population includes a particularly sensitive subpopulation and thus presents a bimodal distribution, the HKAF is determined as the ratio between an upper percentile value of this subpopulation and the central tendency of the general healthy population (IPCS, 2005; Meek *et al.*, 2002).

The magnitude of HKAF for oral exposures has been inferred based on analysis of therapeutic drug database for metabolic pathway-related differences in pharmacokinetic

parameters among healthy adults of different phenotypes, as well as other subpopulations (Dorne *et al.*, 2005; Ginsberg *et al.*, 2002). In absence of experimental data, physiologically-based pharmacokinetic (PBPK) models (or steady-state algorithms) have been used to investigate the adequacy of this factor mostly for the inhalation route and to a limited extent for other routes of exposure (Clewell *et al.*, 2004; Gentry *et al.*, 2002; Mörk and Johanson, 2010; Nong and Krishnan, 2007; Nong *et al.*, 2006; Pelekis *et al.*, 2003).

The risk assessment of some drinking water contaminants (DWCs) considers exposure due to the inhalation of vapors and dermal contact while showering or bathing (CalEPA, 2009; Health Canada 2005, 2006). However, the adequacy of the current use of the point-of-departure and HKAF for one route (oral) to derive the guideline values that accounts for multi-route exposures has not been evaluated. In this regard, data from Clewell *et al.* (2004) suggest that the HKAF may vary depending of the exposure route and the subpopulation considered. Indeed, these authors used a life-stage PBPK approach to evaluate the mean blood concentration of isopropanol (IP) in several age groups and for different exposure routes. For a body weight-adjusted equivalent oral exposure, the ratios of the mean blood concentration of IP between neonates (0–0.5 years) or infants (0.5–5 years), *vs* adults (25 years), were 1.9 and 1.5, respectively. For dermal exposure to IP in water, the corresponding values were 1.1 and 0.9. Finally, for inhalation of 1 ppb, the neonate/adult and toddler/adult rations ratios were 2 and 1.5, respectively.

The study of Clewell *et al.* (2004) evaluated the effect of exposure route on age-specific internal dose for a single water-soluble and poorly volatile chemical (IP) using a deterministic PBPK modeling approach but not a probabilistic one, which would be required to quantify the HKAF under the IPCS approach. Accordingly, the objective of the current study was to assess the effect of the exposure route on the magnitude of the HKAF in different subpopulations, using environmental toxicants possessing various characteristics.

2.2 Methods

The overall method involved using a stochastic PBPK modeling approach to simulate the internal dose metrics in various subpopulations (adults, neonates, elderly and pregnant women), for different chemicals and exposure routes, in order to compute route-specific HKAFs for each chemical on the basis of the IPCS approach.

2.2.1 PBPK model structures and parameters

2.2.1.1 Choice of the PBPK model

For the purpose of simulating various exposure routes, a modified version of the multi-route PBPK model framework published by Haddad *et al.* (2006) was used. The Haddad *et al.* model allows calculation of physiological parameters as a function of four determinants, namely body weight, height, age, and gender. These equations were defined mainly based on the work of Price *et al.* (2003b), used to generate the P^3M database (The Lifeline Group Inc, Annandale, VA). These equations vary according to the age group simulated; hence this model ensures that physiological parameters are correlated for a given body weight/height, age and gender, while authorizing age-specific variations in the relationships between the physiological parameters and their determinants. Apart from adults (18–64 yr), four presumed sensitive subpopulations were chosen for this study, *i.e.* neonates (birth–30 d), children (1–3 yr), elderly (65+ yr), and 38^{th} week pregnant women (PW).

2.2.1.2 Selection of surrogate chemicals and their specific parameters

Four VOCs that occur as DWCs and known for their potential multi-route exposures were chosen (Table 2-I). These chemicals were chosen according to the following criteria: availability of PBPK models and parameters in the literature, variability in the pulmonary

clearance potential (as reflected by the range of blood:air partition coefficients (Pb)) and the variability in biochemical properties (highly/poorly metabolized; generation of reactive or stable metabolites). Chloroform was chosen as a highly-metabolized VOC producing reactive metabolite, with high pulmonary clearance potential (Pb = 7.43). Bromoform was selected as a highly metabolized chemical producing reactive metabolites, with a much lower pulmonary clearance potential (Pb = 102.3). Tetrachloroethylene (PERC) was chosen as a surrogate for poorly metabolized chemicals with low Pb (11.58). To evaluate the impact of the fraction metabolized on the kinetics of resulting circulating metabolite, the fourth and final model substance chosen for this study was trichloroethylene (TCE), a highly metabolized chemical with Pb comparable to PERC (9.2) and producing the same stable metabolite principally cleared in urine, *i.e.* trichloroacetic acid (TCA).

Generally, the parameters related to the chosen VOCs (Table 2-I) were taken from the literature. Others were calculated, such as PERC's oral absorption constant which was determined from mouse data using $BW^{-0.25}$ adjustment method as done by Haddad *et al.* (2006) for the other VOCs. Also, the placenta:blood partition coefficients (PC) were calculated using the approach of Poulin and Krishnan (1995) on the basis of placenta composition data of Klingler *et al.* (2003). Foetus:blood PC was assumed to be the same as for highly perfused tissues.

2.2.1.3 Modifications to the selected PBPK framework

The Haddad *et al.* model was modified to generate the models relevant to the VOCs and subpopulations investigated in this study. These models are conceptually represented in Fig. 2.1 and were written in Microsoft Excel® (Microsoft Corporation, Seattle, WA) as per Haddad *et al.* (1996).

First, a renal compartment was added for chloroform and PERC for which metabolism does occur in kidneys *albeit* at a lower rate than in liver, as well as for bromoform, since it possesses a different partition coefficient than highly perfused tissues (Table 2-I). Second, a foeto-placental unit was added for representing PW. Third, a compartment was added to describe the kinetics of the stable metabolite TCA produced by oxidation of TCE and PERC, and its excretion in urine. The hepatic metabolism term in the Haddad *et al.* model was re-written to facilitate the use of catalytic turnover of CYP2E1 (in pmol/mg of microsomal protein, MSP), which is responsible for the main oxidation pathway of the VOCs investigated in this study, based on the approach of Nong *et al.* (2006). Thus, for a given individual, the maximal rate of metabolism ($Vmax_{adj}$) was recalculated as follows:

$$Vmax_{adj} = \frac{Vmax}{[CYP2E1]_{ad} \times Vl_{ad}} \times [CYP2E1]_{ind} \times Vl_{ind} \quad (1)$$

where $[CYP2E1]_{ad}$ is the mean CYP2E1 hepatic concentration (51.7 pmol/mg of microsomal protein, MSP) in the reference group of 20 adults described by Lipscomb *et al.* (2003), for which the mean body weight (77.25 kg), and corresponding liver volume (Vl_{ad}) were also available. $[CYP2E1]_{ind}$ is the CYP2E1 hepatic concentration of the individual simulated and Vl_{ind} the liver volume. Constant hepatic microsomal protein concentration across the subpopulations was assumed. The impact of this assumption on current study's results may be verified as relevant data become available.

The kidney metabolism for chloroform and PERC was accounted for by adding a metabolism term in the mass-balance equation for this compartment. This term was adjusted according to the relative metabolic activity of kidney as compared to liver (3.3 % for chloroform, 10 % for PERC, Table 2-I). The relative expression of renal CYP2E1 in neonates was assumed to be the same as the neonate (or children)/adult CYP2E1

concentration ratio in the liver. Finally, the rate of production of TCA from PERC and TCE metabolism was calculated as :

$$\frac{dA_{TCA}}{dt} = \frac{Vmax_{tiss} \times C_{V\,tiss}}{Km + C_{V\,tiss}} \times fracmet \qquad (2)$$

where $Vmax_{tiss}$ is the maximum rate of metabolism in tissue (liver or kidney), $C_{V\,tiss}$ is the free concentration of chemical in venous blood of tissue (liver or kidney) and fracmet is the fraction of metabolism that generates TCA. Fracmet was estimated from Soucek *et al.* (1960) for TCE (0.25) (described in Clewell *et al.* (2000)), and taken from Clewell *et al.* (2005) for TCA (0.6). For TCA produced in kidney from the biotransformation of PERC, Eq. 2 translates directly into amount of TCA appearing in urine, while for the amount of TCA produced from PERC in the liver (A_{TCA}), it is diluted in a volume of distribution corresponding to 10 % of total body, before being excreted according to a urinary excretion constant (K_u) (Clewell *et al.*, 2005):

$$\frac{dA_{TCA}}{dt} = \frac{Vmax \times C_{V\,tiss}}{Km + C_{V\,tiss}} \times fracmet - K_u \times A_{TCA} \qquad (3)$$

The K_u value indicated in Table 2-I was applied to adults. K_u values for other subpopulations were determined based on their renal function, relative to adults (Appendix 2a). On the basis of the approach of Clewell *et al.* (2004) for TCA and nicotine, this function was assumed to correspond to the product of (1) the mean volume-adjusted renal blood flow (QK_{adj}, L/min-g of kidney) and (2), the renal extraction ratio (E_{ren}). On the basis of the relative glomerular filtration rate (GFR, L/min-1.73 m^2) and the mean body surface area, E_{ren} was determined using the absolute GFR (L/min), and the mean renal blood flow (Qk, L/min):

$$E_{ren} = \frac{GFR}{(GFR + Qk)} \quad (4)$$

2.2.1.4 Model parameters

The statistics of the physiological determinants defining each subpopulation are indicated in Table 2-II. These include body weight (BW), height (BH), microsomal CYP2E1 concentration in the liver, and GFR. The equations presented in Appendix 2b were used to calculate the PBPK model input parameters (blood flows, alveolar ventilation rates and tissue volumes) that are specific to a given set of BW, BH, and age. The resulting parameter values for an "average" individual of each subpopulation are presented in Table 2-III.

In Table 2-II, BW and BH of adults, children and elderly were based on data extracted from P^3M database (Price *et al.*, 2003b). However, for neonates, BW data were obtained from Johnsrud *et al.* (2003), excluding those with a BW smaller than 2 kg since that would not allow survival outside a neonatology care unit (AM Nuyt, neonatologist, personal communication). BH were estimated from data reported by Nelson (1991). BW and BH for PW were obtained from the P^3M database for non-pregnant women, to which the mean weight gain during the first 38 weeks of pregnancy as per ICRP (2002) (12.5 kg) was added. CYP2E1 hepatic concentration and GFR were taken from the literature (DeWoskin and Thompson, 2008; Faustman and Ribeiro, 1990; Johnsrud *et al.*, 2003; Lipscomb *et al.*, 2003; Sarangapani *et al.*, 2003).

Equations presented in Appendix 2b are essentially the same as those of the Haddad *et al.* model, and would appear to be adequate for evaluating parameter values for infants of 0–

1 year old (Verner *et al.* 2006). Among the differences with the original model, the rest of the body compartment, instead of fat compartment, was considered as the buffer for computing the mass-balance of tissue volumes. Thus, fat compartment volume, as well as kidney volume, was calculated as per Price *et al.* (2003b), except for neonates for whom a value of 14 % of body weight was used, based on data by Haddad *et al.* (2001). Liver volumes in adults and elderly were calculated as 2.6 % of body weight (Brown *et al.*, 1997). The volume of the rest of the body was calculated as the difference between the body weight and the sum of the volumes of the other compartments, including the bones. The latter was calculated as 9 % of BW in adults while in elderly, this fraction was modified according to a factor that reflects the age-related decrease in the ratio of total body bone mineral to lean body mass (Price *et al.*, 2003b). In infants and children, the bone volume was calculated as the difference between the total bone volume and the bone marrow volume, both of which were calculated as per Haddad *et al.* (2001).

All the blood flows were calculated based on tissue volume as per Haddad *et al.* (2006), except for kidney blood flows, which were based on Brown *et al.* (1997) for adults and elderly and from Price *et al.* (2003b) for infants and children. The blood flow for the rest of the body compartment was calculated based on the volume of tongue, heart and muscles. Since there is no concrete evidence that volume of internal organs other than kidneys increase during pregnancy (ICRP, 2002), PW model parameters were calculated using the equations and BW of a non-pregnant adult women, except for kidney volume, which was based on the body weight at 38^{th} week of pregnancy. A factor was added for fat volume, skin blood flow and cardiac output to reflect the increase in these parameters until 38^{th} week of pregnancy (Faustman and Ribeiro, 1990; ICRP, 2002). PW's body surface area was calculated based on the Dubois & Dubois equation, validated for PW by Wang *et al.* (1992). Foeto-placental parameters were based on data from ICRP (2002) and Clewell *et al.* (1999).

2.2.2 Simulation of exposure scenarios

For each subpopulation, three route-specific exposure scenarios representative of low, environmental exposure levels, were defined:

1) 24-hour inhalation of air containing 5 µg/m^3;

2) a bolus oral dose of 1 µg/kg-d; and

3) 30-min dermal contact with water containing 15 µg/L;

Scenario 1 was set to yield an inhaled dose of 1 µg/kg-d (*i.e.* equivalent to scenario 2), assuming a 70 kg adult inhaling 15 m^3/d. Water concentration used in scenario 3 would yield a daily dose of 1 µg/kg-d for a 70 kg adult, as per the risk assessment of Health Canada (2005, 2006) that considers multi-route exposure during a 30 min bath. Also, 75 % of the body surface was assumed to be in contact with water (Lindstrom *et al.*, 1994). For all three scenarios, the computed dose metrics included the 24-h area under the arterial blood concentration *vs* time curve, for either the parent compound (AUC_{pc}) or stable metabolite TCA (AUC_{met}, for TCE and PERC), as well as the amount of parent compound metabolized per 24 h per L of liver (AMET). These dose metrics were chosen preferably to peak blood concentrations as they better reflect the internal dose metrics for chronic exposure scenario of relevance to the derivation of RfD or RfC for which HKAFs are used.

2.2.3 Probabilistic modeling and calculation of route-specific HKAF

Given the variability in the physiology of two persons of identical age and body mass index, variability in some physiological parameters for a given set of BW and BH was authorized during the Monte Carlo (MC) simulations. To do so, a “variability term” (VT), characterized by the distributions indicated in Table 2-II, was added as a multiplier of the

results of selected physiological parameter values calculated with equations in Appendix 2b. As such, the VT for Qp was attributed in order to ensure that its value would correspond to a value ranging between 0.8 and 1.2 times Qc, at resting conditions. The VT for liver blood flow was attributed based on the tissue weight-adjusted liver blood flow data obtained by means of petscan imaging by Taniguchi *et al.* (1996). Based on Thomas *et al.* (1996), the VT for fat blood flow was assumed to correspond to half the VT for liver blood flow. Although the literature data used to define the VT values were available for adults only, these values were also used for describing variability in all subpopulations.

The parameters to which a VT was assigned were selected on the basis of their higher sensitivity indexes (SI) towards AUC_{pc} obtained in a sensitivity analysis. To ensure that no route-specific parameter was omitted in such analysis, the multi-route exposure scenario (during 30 min bathing) was considered for this purpose. This was done using McKone (1987)'s water-to-air transfer model described in Haddad *et al.* (2006) and BW-adjusted water ingestion rates of U.S. EPA (2004). The SI of AUC_{pc} for a given parameter (P) was calculated as:

$$SI = \frac{AUC_{PC\_10} - AUC_{PC\_i}}{P_{10} - Pi} \times \frac{P_i}{AUC_{PC\_i}} \quad (5)$$

where subscript 10 denotes the AUC_{pc} and parameter (P) value when the latter is reduced by 10 % compared to the initial value, indicated by subscript i. The most influential parameters of the PBPK models with respect to the AUC_{pc} in each subpopulation were Ql and Vl (for highly metabolized chemicals only), Qf and Vf (for more lipophilic TCE and PERC), and Qc and Qp for all chemicals (not shown). Thus, with the exception of Vf, all these input parameters were attributed a VT. In the case of the variability in Vf, it depends of the variability of both height and weight, for which PDFs were already determined. These results are coherent with other sensitivity analyses performed for VOCs sharing

similar properties (Bois *et al.*, 1990; Clewell *et al.*, 1994; Nong *et al.*, 2006, Tardif *et al.*, 2002).

For each subpopulation and exposure scenario, MC simulations were performed using Crystal Ball software (Oracle™, Redwood Shores, CA) in order to generate PDF for internal dose metrics following 2000 iterations. To avoid unrealistic combinations, BW and BH were correlated to 60 % based on population distribution of body mass index in Canada (Statistics Canada, 2003). Except for PW, a "yes or no"-type variable with equal probability (0.5 each) was set so that the male or female equations in Appendix 2b would be chosen for each MC iteration. The dose metric PDFs for males and females resulting from the MC simulations were thus combined (except for PW) to calculate the route-specific HKAF as the ratio of the 95th percentile value in subpopulations to the median in adults.

2.3 Results

The models used in the current study generated pharmacokinetic profiles that were consistent with the simulations obtained with the original Haddad *et al.* model (Fig. 2.2) for a 70 kg male adult. Because the same Pb values were used as in the Haddad *et al.* model for TCE but not for chloroform, better agreement was obtained for the former than the latter. When the gender, mean age, body weight and height data of volunteers involved were entered as input data along with experimental conditions for chloroform (Lévesque *et al.*, 2002), TCE (Mueller *et al.*, 1975) and PERC (Fernandez *et al.*, 1976; Volkel *et al.*, 1998), the model predicted reasonably well the kinetics of parent compounds (2.IIIa-b) and TCA (Fig. 2.3c).

2.3.1 Simulation of route-specific internal dosimetry and corresponding HKAFs

Since the differences in the value of the kinetics determinants between male and female were small using equations in Appendix 2b (see Table 2-III), simulations for the three route-specific exposure scenarios, and using chloroform as an example, are presented for the average male in Fig. 2.4. Route-specific HKAFs are described hereafter and mean values of the dose metrics simulated in each subpopulation for the various scenarios are reported in Appendix 2c.

2.3.1.1 Inhalation exposure

AUC_{pc}-based HKAF are greater in neonates as compared to the other subpopulations for the four VOCs investigated (Table 2-IV). The HKAF exceeds the default value in the case of bromoform (3.6). The lowest HKAF in neonates is obtained for PERC (1.8), followed by chloroform (2.1) and TCE (2.2). This sequence in the chemical-specific HKAFs is repeated within each subpopulation. On the basis of AMET, the most sensitive subpopulation varies according to the chemical: children for chloroform and TCE (HKAF = 1.6 and 1.5 respectively), neonates for bromoform (2.0) and PW for PERC (2.2). Greatest values per subpopulation are obtained for PERC. Elderly and neonates present highest AUC_{met}-based HKAFs for PERC (2.2) and TCE exposure (2.1), respectively.

2.3.1.2 BW-adjusted oral exposure

BW-adjusted oral exposure to 1 µg/kg-d generates the highest HKAFs in neonates (Table 2-V). These exceed the default value in the case of bromoform (7.4) and chloroform (4.9), while the lowest value is observed for PERC (1.4), thus this sequence is the same as for inhalation exposure. Based on AMET, greater HKAFs are obtained in PW for chloroform, bromoform and TCE (1.5), and in elderly for PERC (2.1). Within each

subpopulation, greatest HKAF for AMET is obtained for PERC. Finally, on the basis of AUC_{met}, HKAFs are greater for the elderly following PERC exposure (2.2).

2.3.1.3 Dermal exposure

Equally as for the other exposure route, AUC_{pc}-based HKAF are greater in neonates as compared to the other subpopulations, with values (2.6–4.4) slightly greater than for inhalation exposure but lower than for the BW-adjusted oral exposure scenario (Table 2-VI). The HKAF, in all cases, is greater for bromoform and exceeds 3.16 in neonates (4.4). On the basis of AMET, greatest HKAFs are obtained in children for chloroform (1.8), TCE (1.8) and PERC (2.4), while for bromoform, the highest value is obtained in neonates (2.4). Finally, greater AUC_{met}-based HKAF is observed based on simulations of TCE (2.7) and PERC (2.9) kinetics in neonates.

2.4 Discussion

The present work aimed at evaluating the effect of the exposure route on HKAF. While several multi-route PBPK models have been published in the literature (e.g. Clewell *et al.*, 2004; Tan *et al.*, 2007), the Haddad *et al.* (2006) model used here was of particular interest for the current study since it contains age- and gender- specific equations to define physiological parameters of the populations of interest. This feature of the chosen PBPK model was relevant to the simulation of interindividual variability in the toxicokinetics of chemicals given that the proportion of body weight attributed to each tissue varies depending of the age (Clewell *et al.*, 2002; Haddad *et al.*, 2001). The coherence of the parameter values in Table 2-III with expected average values of each subpopulation (Clewell *et al.*, 2002; ICRP, 2002; Price *et al.*, 2003b) enhances the confidence in their use

in probabilistic models to evaluate the interindividual toxicokinetic differences relevant to HKAF, as done in the present study.

The results obtained suggest that for each subpopulation, the HKAF varies as a function of the chemical, exposure route and dose metrics, but exceedence of the default value for HKAF (3.16) was seldom observed, *i.e.* on the basis of AUC_{pc} in neonates only for bromoform for all exposure routes (range: 3.6-7.4) as well as for oral exposure to chloroform (4.9). The exceedence in these cases can be explained by the fact that the rate of CYP2E1-mediated trihalomethane metabolism and the extraction ratio are lower in neonates compared to adults. Since the intrinsic metabolic clearance is high (Table 2-I) and a highly sensitive parameter of the PBPK models for chloroform and bromoform (not shown), the decrease in enzyme content and intrinsic clearance in neonates compared to adults leads to the marked difference in blood concentration, AUC_{pc} and HKAFs. In other words, the exceedence of default HKAFs in these cases is likely a consequence of perfusion-limited clearance in adults becoming enzyme-limited clearance in neonates, an effect that becomes conspicuous for oral exposures (due to first-pass effect) and particularly for neonates at the tail end of the distribution (e.g., 95^{th} percentile). The neonate-adult differences in AUC_{pc} of chloroform and bromoform simulated for a single oral dose in the present study could also be influenced by other kinetic parameters such as the volume of distribution and the oral absorption rate, factors which are unlikely to influence the magnitude of HKAF during repeated/chronic exposures (leading to steady-state). It is noteworthy that the chronic adverse effects underlying the RfD or RfC of the VOCs investigated in the present study are attributed to the metabolites (ATSDR 1997a-c, 2005; U.S. EPA, 2010). In this context, exceeding the default factor on the basis of AUC_{pc}, is less of a concern as compared to exceedance on the basis of AMET or AUC_{met}, which has not occurred in the present study. Overall then, the observed exceedances should not be interpreted as evidence of inadequacy of the default value for the investigated substances, but rather as a basis for evaluating its appropriateness in light of the current knowledge of the mode of action and nature of the toxic moiety (*i.e.*, parent chemical *vs* metabolite). The

results of this study (Tables 2-IV to 2-VI) show that many of the computed values of HKAF are below 3.16, implying that the default factor may overestimate the risk for most sensitive subpopulations. These results might also be interpreted as proof of the adequacy of the default HKAF for its capacity to protect these subpopulations.

The results obtained also suggest that using the same HKAF default value regardless of the exposure route and chemical when establishing RfD and RfC, as well as DWC guidelines for chemicals presenting potential for multi-route exposure, should be rethought. This then raises the questions of whether the default HKAF is adequate to account for interindividual variability of internal dose during multi-route (aggregate) exposure, and whether the default HKAF is appropriate in such conditions. Presumably, variability of internal dose during multi-route exposures lies within the range of the variabilities associated with each exposure route taken separately, in accordance with the principle of the propagation of error (Thomas *et al.* 1996). Moreover, the interindividual variability in internal dose for multi-route exposures would, in part, be due to variability in the intake of drinking water. Whether the development of DW guidelines based on average adult's multiroute exposure (expressed as litre-equivalents (CalEPA, 2009; Health Canada 2005, 2006)), is also sufficiently protective of other subpopulations remains to be evaluated. For example, the higher ventilation rate and dermal surface area in PW might lead to greater absorbed dose of some DWCs after a shower or bath compared to adults and in such cases, the litre-equivalents and/or HKAF used in an assessment may not be adequate to cover specific subpopulations such as PW.

The results of the current study are comparable to route-specific HKAFs reported in previous probabilistic and deterministic studies. Indeed, the HKAFs for the inhalation route obtained in this study are comparable to the adult - child factors obtained for CYP2E1 substrates by Nong *et al.* (2006) (for toluene), and Pelekis *et al.* (2003) (for

dichloromethane), using a probabilistic methodology. For example, Nong *et al.* (2006) obtained values varying between 2.5 and 3.9 for neonates on the basis of AUC_{pc} of toluene whereas values varying between 1.8 and 3.6, depending on the chemical, were obtained in the current study. Adult - child factors of 1.5 and approximately 2.3 were obtained respectively for children aged 1-11 or 1-5 years by Nong *et al.* (2006) and Pelekis *et al.* (2003), while corresponding value varied between 1.6 and 2.0 for children aged 1-3 years in the current study. Based on distributions of AUC_{pc} reported by Mörk and Johanson (2010), HKAF for inhaled acetone (a chemical not evaluated in the present study) are as follows: 1.5 in adults, 2.5 in 1-year children and 2.4 in 3-month-old babies.

The neonate (or child)/adult ratios of median internal dose for the inhalation route obtained in this study are comparable to those obtained by Price *et al.* (2003a) for furan and Clewell *et al.* (2004) for isopropanol, using a deterministic PBPK model. Actually, current study yielded ratios based on AUC_{pc} varying between 1.7 and 2.2 in neonates and between 1.3 and 1.5 in children, depending upon the substance (see Appendix 2c). These values are similar to Clewell et al (2004)'s values for neonates (2.0) as well as those reported by Clewell *et al.* (2004) and Price *et al.* (2003a) for children (1.6 and 1.5, respectively). The consistency of the observations can be explained by the fact that the chemicals investigated either share the same enzyme-mediated (*i.e.*, CYP2E1) metabolic pathway (Price *et al.* (2003b) or are metabolized via a pathway (ADH) presenting a comparable child-to-adult ratio of hepatic enzyme concentration/activity (Sarangapani *et al.* 2003).

For the oral route, the only relevant comparison with published literature on HKAF could be made with the values of 1.9 and 1.5 for isopropanol reported by Clewell *et al.* (2004) for neonates and children, which are similar to our results for neonates (range: 1.2–2.9, Appendix 2C) but slightly higher than the values obtained for children (range: 1–1.1). For the dermal route however, Clewell *et al.* (2004)'s values are lower than the values obtained

in the present study, likely due to isopropanol's poor liposolubility possibly limiting its dermal penetration and internal dose variability.

For both inhalation and oral exposures, the results of the present study can be compared to that of Sweeney *et al.* (2003) and Kirman et al (2005; 2008) for acrylonitrile (ACN). The variability in AUC_{pc} allowed Kirman *et al.* (2005; 2008) to estimate the ratio [95^{th} percentile/mean], which varied between 1.8 and 2.2. This value is comparable to adult's AUC_{pc}-based HKAF obtained in the current study for bromoform (1.5–2.1), a chemical that similar to ACN exhibits both high hepatic extraction ratio and Pb value.

Current study's results are interpretable within the limits of the underlying assumptions. First, the HKAF is assumed to correspond to the ratio of 95^{th} percentile/median of the internal dose. Based on regulatory requirements, other statistics (99^{th} percentile, mean, etc.) may be used. Second, current study is based on the consideration of some subpopulations that are generally identified as being the sensitive ones in the literature (neonates, children, pregnant women, elderly). However, this did not include other subpopulations such as health-impaired children and elderly. Third, the dose metrics chosen are assumed to be reasonably adequate indicators of the interindividual variability in toxicokinetics during chronic exposures.

Conceptually, differences between the subpopulations in the values of the determinants of toxicokinetics could translate into corresponding differences in dose metrics and ultimately, contribute to explain the chemical-, subpopulation-, dose metric-, and route- specific HKAF. Route-specific sensitivity analyses would allow to assess the extent to which the different assumptions and model parameters affect the dose metric predictions, for each exposure route and chemical. This, along with variability analyses such as those performed

by Sweeney *et al.* (2003), can help define the key sources of uncertainty and their impact on the overall outcome, *i.e.* the value of the HKAF. The results of such concerted efforts can help define a decision tree or a priority scheme to identify key determinants for computing HKAFs in a chemical, route and dose-metric specific context. For example, for a given combination of chemical, route and dose metrics, specific parameters (e.g. delivery rate, intrinsic clearance) could explain the extent of the variability and magnitude of HKAF (Fig. 2.4, Tables 2-IV to 2-VI). As discussed below, apart from route-specific intake rate in the various subpopulations, current study's results bring out the importance of considering the interindividual differences in the key determinants of toxicokinetics (e.g., Qp, Pb, CLint, Ql, renal clearance) when estimating the HKAF.

Greater inhalation and dermal intake on a BW basis in neonates as compared to other subpopulations would appear to explain their corresponding greater AUC_{pc}-based HKAFs (Tables 2-IV, 2-VI), but not for the BW-adjusted equivalent oral exposure, where the reduced metabolic clearance in neonates would account for the results (e.g., Nong *et al.*, 2006, Valcke and Krishnan, 2009) (Table 2-V). BW-adjusted values for Qp and BSA calculated from Table 2-III are 11 L/h-kg and 623 cm^2/kg in male neonates as compared to 5.1 L/h-kg and 242 cm^2/kg in male adults, respectively. Also, greater-than-adult alveolar ventilation rate in PW (Brochu *et al.*, 2006; 6.82 L/h-kg from Table 2-III) translates into greater-than-adult inhalation HKAFs and equally, greater pulmonary clearance thus lower-than-adults dermal HKAFs. With regard to Pb, it influences the net inhalation intake for each chemical, as well as their pulmonary clearance regardless of the exposure route. Thus, its value approximately 10 times greater for bromoform than the three other chemicals results in overcoming the potential for pulmonary clearance by respiratory loss that is expected for low-Pb chemicals, because high-Pb chemicals tends to remain in blood (Poulin and Krishnan, 1996), regardless of the ventilation rate. Since pulmonary ventilation is greater in neonates/children as compared to adults (see Table 2-III), the neonate (or children)/adult difference in AUC_{pc} is greater for bromoform than for the other chemicals, resulting in greater HKAFs for every exposure scenarios (Tables 2-IV to 2-VI).

Regarding the influence of hepatic extraction ratio on HKAFs, the greatest AMET-based values were obtained for inhalation and dermal exposures for the subpopulation presenting the greatest intake on a BW-basis for chemicals with a high intrinsic clearance, *i.e.* the children aged 1–3 yr, for chloroform and TCE, and neonates for bromoform (Tables 2-IV to 2.VI). Conversely, low hepatic CYP2E1 levels in neonates aged 0-30 days (Johnsrud *et al.*, 2003) is a likely explanation for their smallest AMET-based HKAFs regardless of exposure route in the case of PERC (range: 0.9–1.9), exhibiting enzyme-limited metabolism. For BW-adjusted oral exposure, PW are exposed to higher absorbed doses leading to greater AMET-based HKAFs compared to other subpopulations for highly metabolized chemicals such as chloroform, bromoform and TCE.

Along with the determinants of the AMET-based HKAFs, the differences in the renal clearance exhibited by each subpopulation contribute to explain the trends observed in AUC_{met}-based HKAF for TCE and PERC. For BW-adjusted exposure, elderly present the greatest AUC_{met}-based HKAF (Table 2-V) because of 1) their efficient metabolism to TCA due to high hepatic CYP2E1 levels; and 2) their reduced renal clearance of the TCA formed (Sarangapani *et al.* 2003). In turn, the neonates present the highest AUC_{met}-based HKAF for inhalation (Table 2-IV) and dermal exposure (Table 2-VI) to TCE due to 1) greater intake on a BW-basis and 2) a reduced renal clearance (DeWoskin and Thompson, 2008) of the TCA formed from the perfusion-limited metabolism of TCE. Because PERC's metabolism is enzyme-limited, low levels of hepatic CYP2E1 in neonates preclude extensive formation of TCA even if the intake of parent compound is high; they thus may not always represent the most sensitive subpopulation. Since TCA is more effectively cleared by renal excretion in PW (Faustman and Ribeiro, 1990; ICRP, 2002), they exhibit low AUC_{met}-based HKAFs for all exposure regimens.

In conclusion, this work has confirmed that probabilistic PBPK modeling approach is a useful tool for estimating chemical-specific HKAF. In this regard, the route-dependency of HKAF has been, for the first time to our knowledge, systematically elucidated. This dependency is characteristic of the sensitive subpopulation which in turn, has been shown to vary according to the chemical and dose surrogate considered. Finally this study has pointed out the critical role of Pb and hepatic extraction ratio in determining the value of the route-specific HKAF.

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2.5 References

ATSDR. Toxicological Profile for Chloroform. 1997a. Agency for Toxic Substances and Disease Registry, Atlanta GA.

ATSDR. Toxicological Profile for Tetrachloroethylene. 1997b. Agency for Toxic Substances and Disease Registry, Atlanta GA.

ATSDR. Toxicological Profile for Trichloroethylene. 1997c. Agency for Toxic Substances and Disease Registry, Atlanta GA.

ATSDR. Toxicological Profile for Bromoform and Dibromochloromethane. 2005. Agency for Toxic Substances and Disease Registry, Atlanta GA.

Bois, F. Y., Zeise, L., Tozer, T. N. 1990. Precision and sensitivity of pharmacokinetic models for cancer risk assessment: tetrachloroethylene in mice, rats, and humans. *Toxicol Appl. Pharmacol. 102*, 300-315.

Brochu, P., Ducré-Robitaille, J. F., Brodeur, J. 2006. Physiological daily inhalation rates for free-living pregnant and lactating adolescents and women aged 11 to 55 years, using data from doubly labelled water measurements for use in health risk assessment. *Hum Ecol Risk Assess 12*, 702-735.

Brown, R. P., Delp, M. D., Lindstedt, S. L., Rhomberg, L. R., Beliles, R. P. 1997. Physiological parameter values for physiologically based pharmacokinetic models. *Toxicol Ind. Health. 13*, 407-484.

CalEPA. 2009. Public Health Goal for Chemicals in Drinking Water - Trichloroethylene. Pesticide and Environmental Toxicology Branch. Office of Environmental Health Hazard Assessment, California Environmental Protection Agency. Sacramento CA

Clewell, H. J., Gearhart, J. M., Gentry, P. R., Covington, T. R., VanLandingham, C. B., Crump, K. S., Shipp, A. M. 1999. Evaluation of the uncertainty in an oral reference dose for methylmercury due to interindividual variability in pharmacokinetics. *Risk Anal. 19*, 547-558.

Clewell, H. J., III., Gentry, P. R., Covington, T. R., Gearhart, J. M. 2000. Development of a physiologically based pharmacokinetic model of trichloroethylene and its metabolites for use in risk assessment. *Environ Health Perspect. 108*, 283-305.

Clewell, H. J., Gentry, P. R., Covington, T. R., Sarangapani, R., Teeguarden, J. G. 2004. Evaluation of the potential impact of age- and gender-specific pharmacokinetic differences on tissue dosimetry. *Toxicol Sci. 79*, 381-393.

Clewell, H. J., Gentry, P. R., Kester, J. E., Andersen, M. E. 2005. Evaluation of physiologically based pharmacokinetic models in risk assessment: an example with perchloroethylene. *Crit Rev Toxicol. 35*, 413-433.

Clewell, H. J., III., Lee, T. S., Carpenter, R. L. 1994. Sensitivity of physiologically based pharmacokinetic models to variation in model parameters: methylene chloride. *Risk Anal. 14*, 521-531.

Clewell, H. J., Teeguarden, J., McDonald, T., Sarangapani, R., Lawrence, G., Covington, T., Gentry, R., Shipp, A. 2002. Review and evaluation of the potential impact of age- and gender-specific pharmacokinetic differences on tissue dosimetry. *Crit Rev Toxicol. 32*, 329-389.

Corley, R. A., Mendrala, A. L., Smith, F. A., Staats, D. A., Gargas, M. L., Conolly, R. B., Andersen, M. E., Reitz, R. H. 1990. Development of a physiologically based pharmacokinetic model for chloroform. *Toxicol. Appl. Pharmacol. 103*, 512-527.

DeWoskin, R. S., Thompson, C. M. 2008. Renal clearance parameters for PBPK model analysis of early lifestage differences in the disposition of environmental toxicants. *Regul. Toxicol Pharmacol. 51*, 66-86.

Dorne, J. L., Walton, K., Renwick, A. G. 2005. Human variability in xenobiotic metabolism and pathway-related uncertainty factors for chemical risk assessment: a review. *Food Chem. Toxicol. 43*, 203-216.

Dorne, J. L., Renwick, A. G. 2005. The refinement of uncertainty/safety factors in risk assessment by the incorporation of data on toxicokinetic variability in humans. *Toxicol. Sci. 86*, 20-26.

Dourson, M. L., Felter, S. P., Robinson, D. 1996. Evolution of science-based uncertainty factors in noncancer risk assessment. *Regul. Toxicol. Pharmacol. 24*, 108-120.

Dourson, M. L., Stara, J. F. 1983. Regulatory history and experimental support of uncertainty (safety) factors. *Regul. Toxicol. Pharmacol. 3*, 224-238.

Faustman, E. M., Ribeiro, P. 1990. Pharmacokinetic consideration in developmental toxicity. IN: Hood, R.D. (Ed.), *Developmental toxicology: risk assessment and the future*. Van Nostrand Reinhold, New York NY, pp. 109-136.

Fernandez, J., Guberan, E., Caperos, J. 1976. Experimental human exposures to tetrachloroethylene vapor and elimination in breath after inhalation. *Am Ind. Hyg. Assoc. J.* 37, 143-150.

Gargas, M. L., Burgess, R. J., Voisard, D. E., Cason, G. H., Andersen, M. E. 1989. Partition coefficients of low-molecular-weight volatile chemicals in various liquids and tissues. *Toxicol Appl. Pharmacol. 98*, 87-99.

Gearhart, J. M., Mahle, D. A., Greene, R. J., Seckel, C. S., Flemming, C. D., Fisher, J. W., Clewell, H. J., III. 1993. Variability of physiologically based pharmacokinetic (PBPK) model parameters and their effects on PBPK model predictions in a risk assessment for perchloroethylene (PCE). *Toxicol. Lett. 68*, 131-144.

Gentry, P. R., Hack, C. E., Haber, L., Maier, A., Clewell, H. J., III. 2002. An approach for the quantitative consideration of genetic polymorphism data in chemical risk assessment: examples with warfarin and parathion. *Toxicol. Sci. 70*, 120-139.

Ginsberg, G., Hattis, D., Sonawane, B., Russ, A., Banati, P., Kozlak, M., Smolenski, S., Goble, R. 2002. Evaluation of child/adult pharmacokinetic differences from a database derived from the therapeutic drug literature. *Toxicol. Sci. 66*, 185-200.

Haddad, S., Pelekis, M., Krishnan, K. 1996. A methodology for solving physiologically based pharmacokinetic models without the use of simulation softwares. *Toxicol. Lett. 85*, 113-126.

Haddad, S., Restieri, C., Krishnan, K. 2001. Characterization of age-related changes in body weight and organ weights from birth to adolescence in humans. *J Toxicol. Environ Health A. 64*, 453-464.

Haddad, S., Tardif, G. C., Tardif, R. 2006. Development of physiologically based toxicokinetic models for improving the human indoor exposure assessment to water contaminants: trichloroethylene and trihalomethanes. *J Toxicol. Environ Health A*. 69, 2095-2136.

Health Canada. 2005. Guidelines for Canadian Drinking Water Quality: Supporting Documentations - Trichloroethylene. Water Quality and Health Bureau, Healthy Environments and Consumer Safety Branch, Health Canada, Ottawa, ON.

Health Canada. 2006. Guidelines for Canadian Drinking Water Quality: Supporting Documentations - Trihalomethanes. Water Quality and Health Bureau, Healthy Environments and Consumer Safety Branch, Health Canada, Ottawa, ON.

ICRP. 2002. ICRP Publication 89. *Ann ICRP. 32*, 5-265.

IPCS. 1994. Assessing human health risks of chemicals: Derivation of guidance values for health based exposure limits. Environmental Health Criteria 170. International Panel on Chemical Safety, World Health Organization, Geneva, Switzerland.

IPCS. 2005. Chemical-Specific Adjustment Factors (CSAFs) for Interspecies Differences and Human Variability: Guidance Document for the Use of Data in Dose/Concentration-Response Assessment. WHO/IPCS/01.4, 1-96. International Panel on Chemical Safety, World Health Organisation, Geneva, Switzerland

Johnsrud, E. K., Koukouritaki, S. B., Divakaran, K., Brunengraber, L. L., Hines, R. N., McCarver, D. G. 2003. Human hepatic CYP2E1 expression during development. *J Pharmacol. Exp. Ther. 307*, 402-407.

Kirman, C. R., Gargas, M. L., Marsh, G. M., Strother, D. E., Klaunig, J. E., Collins, J. J., Deskin, R. 2005. Cancer dose-response assessment for acrylonitrile based upon rodent brain tumor incidence: use of epidemiological, mechanistic, and pharmacokinetic support of nonlinearity. *Regul. Toxicol. Pharmacol. 43*, 85-103.

Kirman, C. R., Sweeney, L. M., Gargas, M. L., Strother, D. E., Collins, J. J., Deskin, R. 2008. Derivation of noncancer reference values for acrylonitrile. *Risk Anal. 28*, 1375-1393.

Klingler, M., Demmelmair, H., Larque, E., Koletzko, B. 2003. Analysis of FA contents in individual lipid fractions from human placental tissue. *Lipids. 38*, 561-566.

Lévesque, B., Ayotte, P., Tardif, R., Ferron, L., Gingras, S., Schlouch, E., Gingras, G., Levallois P., Dewailly, E. 2002. Cancer risk associated with household exposure to chloroform. *J Toxicol. Environ Health A. 65*, 489-502.

Lindstrom, A. B., Highsmith, V. R., Buckley, T. J., Pate, W. J., Michael, L. C. 1994. Gasoline-contaminated ground water as a source of residential benzene exposure: a case study. *J Expo. Anal. Environ Epidemiol. 4*, 183-195.

Lipscomb, J. C., Teuschler, L. K., Swartout, J., Swan, G. E., Snawder, J. E. 2003. Variance of microsomal protein and cytochrome P450 2E1 and 3A forms in adult human liver. *Toxicol. Mech. Meth. 13*, 45-51.

McKone, T. E. 1987. Human exposure to volatile organi compounds in household tapwater: the indoor inhalation pathway. *Environ sci. technol. 21*, 1194-1201.

Meek, M. E., Renwick, A., Ohanian, E., Dourson, M., Lake, B., Naumann, B. D., Vu, V. 2002. Guidelines for application of chemical-specific adjustment factors in dose/concentration-response assessment. *Toxicology. 181-182*, 115-120.

Mörk, A.K., Johanson, G., 2010. Chemical-specific adjustment factors for intraspecies variability of acetone toxicokinetics usaing a probabilistic approach. *Toxicol. Sci. 116*, 336-348.

Muller, G., Spassovski, M., Henschler, D. 1975. Metabolism of trichloroethylene in man. III. Interaction of Trichloroethylene and Ethanol. *Arch. Toxicol. 33*, 173-189.

Nelson, W. E. 1991. Textbook of Pediatrics, W.B. Sauders Company.

Nong, A., Krishnan, K. 2007. Estimation of interindividual pharmacokinetic variability factor for inhaled volatile organic chemicals using a probability-bounds approach. *Regul. Toxicol. Pharmacol. 48*, 93-101.

Nong, A., McCarver, D. G., Hines, R. N., Krishnan, K. 2006. Modeling interchild differences in pharmacokinetics on the basis of subject-specific data on physiology and hepatic CYP2E1 levels: a case study with toluene. *Toxicol. Appl. Pharmacol. 214*, 78-87.

Pelekis, M., Nicolich, M. J., Gauthier, J. S. 2003. Probabilistic framework for the estimation of the adult and child toxicokinetic intraspecies uncertainty factors. *Risk Anal. 23*, 1239-1255.

Poulin, P., Krishnan, K. 1995. A biologically-based algorithm for predicting human tissue: blood partition coefficients of organic chemicals. *Hum. Exp. Toxicol. 14*, 273-280.

Poulin, P., Krishnan, K. 1996. A mechanistic algorithm for predicting blood:air partition coefficients of organic chemicals with the consideration of reversible binding in hemoglobin. *Toxicol. Appl. Pharmacol. 136*, 131-137.

Price, K., Haddad, S., Krishnan, K. 2003a. Physiological modeling of age-specific changes in the pharmacokinetics of organic chemicals in children. *J Toxicol. Environ Health A. 66*, 417-433.

Price, P. S., Conolly, R. B., Chaisson, C. F., Gross, E. A., Young, J. S., Mathis, E. T., Tedder, D. R. 2003b. Modeling interindividual variation in physiological factors used in PBPK models of humans. *Crit Rev Toxicol. 33*, 469-503.

Rao, H.V., Brown, R. 1993. A Physiologically-based Pharmacokinetic Model Assessment of Tetrachloroethylene in Groundwater for a Bathing and Showering Determination. *Risk Anal. 17*, 583-598.

Sarangapani, R., Gentry, P. R., Covington, T. R., Teeguarden, J. G., Clewell, H. J., III. 2003. Evaluation of the potential impact of age- and gender-specific lung morphology and ventilation rate on the dosimetry of vapors. *Inhal. Toxicol 15*, 987-1016.

Soucek, B., Vlachova, D. 1960. Excretion of trichloroethylene metabolites in human urine. *Br. J Ind. Med. 17*, 60-64.

Statistics Canada. 2003. Canadian community health survey, cycle 2.1. http://www.statcan.gc.ca/cgi-bin/imdb/p2SV.pl?Function=getSurvey&SurvId=3226&SurvVer=0&SDDS=3226&InstaId=15282&InstaVer=2&lang=en&db=imdb&adm=8&dis=2. Last accessed: February 1st 2010.

Sweeney, L. M., Gargas, M. L., Strother, D. E., Kedderis, G. L. 2003. Physiologically based pharmacokinetic model parameter estimation and sensitivity and variability analyses for acrylonitrile disposition in humans. *Toxicol. Sci. 71*, 27-40.

Tan, Y. M., Liao, K. H., Clewell, H. J., III. 2007. Reverse dosimetry: interpreting trihalomethanes biomonitoring data using physiologically based pharmacokinetic modeling. *J Expo. Sci. Environ Epidemiol. 17*, 591-603.

Taniguchi, H., Oguro, A., Koyama, H., Masuyama, M., Takahashi, T. 1996. Analysis of models for quantification of arterial and portal blood flow in the human liver using PET. *J Comput. Assist. Tomogr. 20*, 135-144.

Tardif, R., Droz, P. O., Charest-Tardif, G., Pierrehumbert, G., Truchon, G. 2002. Impact of human variability on the biological monitoring of exposure to toluene: I. Physiologically based toxicokinetic modelling. *Toxicol Lett. 134*, 155-163.

Thomas, R. S., Bigelow, P. L., Keefe, T. J., Yang, R. S. 1996. Variability in biological exposure indices using physiologically based pharmacokinetic modeling and Monte Carlo simulation. *Am Ind. Hyg. Assoc. J. 57*, 23-32.

U.S. EPA. 2002. A review of the reference dose and reference concentration process. Risk Assessment Forum. EPA/630/P-02/00F. Washington DC.

U.S. EPA. 2004. Estimated Per Capita Water Ingestion and Body Weight in the United States-An Update. Office of science and technology, Office of water. EPA-822-R-00-001. Washington DC 20460, U.S. Environmental Protection Agency.

U.S. EPA. 2010. Integrated Risk Information System. http://cfpub.epa.gov/ncea/iris/index.cfm?fuseaction=iris.showSubstanceList. Last accessed: February 1st 2010.

Valcke, M., Krishnan, K. 2009. Physiologically Based Pharmacokinetic Modeling in the Risk assessment of Developmental Toxicants. IN: Hansen, D.K. Abbott, B.D. (Eds.) *Developmental Toxicology,* Informa Health Care, New York NY, pp. 243-274.

Verner, M. A., Ayotte, P., Muckle, G., Charbonneau, M., Haddad, S. 2009. A physiologically based pharmacokinetic model for the assessment of infant exposure to persistent organic pollutants in epidemiologic studies. *Environ. Health Perspect. 117*, 481-487.

Volkel, W., Friedewald, M., Lederer, E., Pahler, A., Parker, J., Dekant, W. 1998. Biotransformation of perchloroethene: dose-dependent excretion of trichloroacetic acid, dichloroacetic acid, and N-acetyl-S-(trichlorovinyl)-L-cysteine in rats and humans after inhalation. *Toxicol Appl. Pharmacol. 153*, 20-27.

Wang, Y., Moss, J., Thisted, R. 1992. Predictors of body surface area. *J Clin Anesth. 4*, 4-10.

FIGURE CAPTIONS

Figure 2.1 : Conceptual representation of the multi-route PBPK models for chloroform, bromoform, TCE and PERC. Abbreviations : K, constants for Michaelis-Menten kinetics (m), oral absorption (or), dermal permeability coefficient (p), urinary excretion (u); Pb, blood:air partition coefficient; Qc, cardiac output; Qp, alveolar ventilation rate; Q, blood flows to fat (f), highly perfused tissues (h), rest of the body (r), skin (sk), liver (l), kidney (k) placenta (pl) and foetus (fet); Vmax: maximum velocity of metabolism. Lines represent the original PBPK framework from Haddad *et al.* (2006) while dotted lines represent the modifications introduced in order to derive chemical or subpopulation-specific PBPK models.

Figure 2.2 : Evaluation of the impact of the modifications to the original Haddad *et al.* (2006)'s model on the resulting predictions. The 24-h simulations of arterial blood concentration of chloroform and TCE performed by the modified model (black lines) are compared to the same simulations obtained with the Haddad *et al.* (2006)'s model (grey lines), for a 70 kg adult exposed 12 hours to 1 ppm in air.

Figure 2.3 : Evaluation of the modified PBPK model. a) Comparison of the kinetics of chloroform as simulated by the modified model with the experimental data following inhalation and dermal exposure during a 10 min shower (mean ± SD) as reported by Lévesque *et al.* (2002). b) Comparison of the kinetics of TCE and PERC as simulated by the modified model with the experimental data following inhalation exposure to TCE (mean ± SEM) reported by Muller *et al.* (1975), and to PERC (mean) reported by Fernandez *et al.* (1976). c) Comparison of the kinetics of TCA as simulated by the modified model with the experimental data following exposure to TCE (mean ± SEM)

reported by Muller *et al.* (1975), and to PERC (mean ± SD) reported by Volkel *et al.* (1998) (mean, no SD available for 10 ppm exposure).

Figure 2.4 : Model simulations of the arterial blood concentration of chloroform in average male adult, child and neonate, as well as pregnant woman (PW), for three route-specific exposure scenarios. A) 24-h inhalation of 5 µg/m^3; B) BW-adjusted oral exposure to a bolus 1 µg/kg-d dose; C) 30-minute dermal exposure of 75 % of the body surface area through water containing 15 µg/L of chloroform. Pharmacokinetic profiles in elderly are indistinguishable from the adult and thus they were omitted.

Figure 2.1

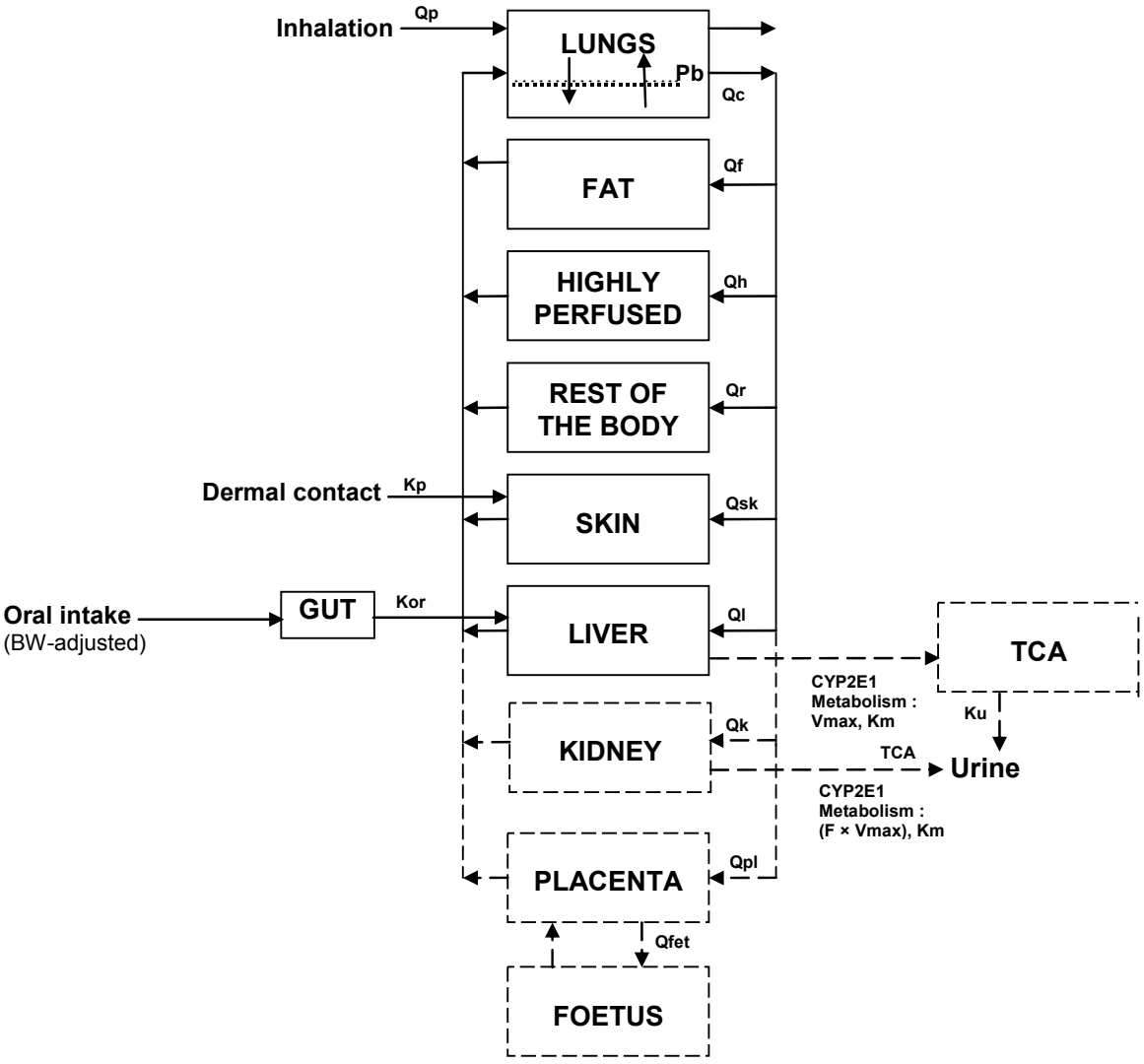

Inhalation
Qp
LUNGS
Pb
Qc
FAT
Qf
HIGHLY PERFUSED
Qh
REST OF THE BODY
Qr
Dermal contact
Kp
SKIN
Qsk
Oral intake
(BW-adjusted)
GUT
Kor
LIVER
Ql
TCA
CYP2E1 Metabolism : Vmax, Km
Ku
Qk
KIDNEY
TCA
Urine
CYP2E1 Metabolism : (F × Vmax), Km
PLACENTA
Qpl
Qfet
FOETUS

Figure 2.2

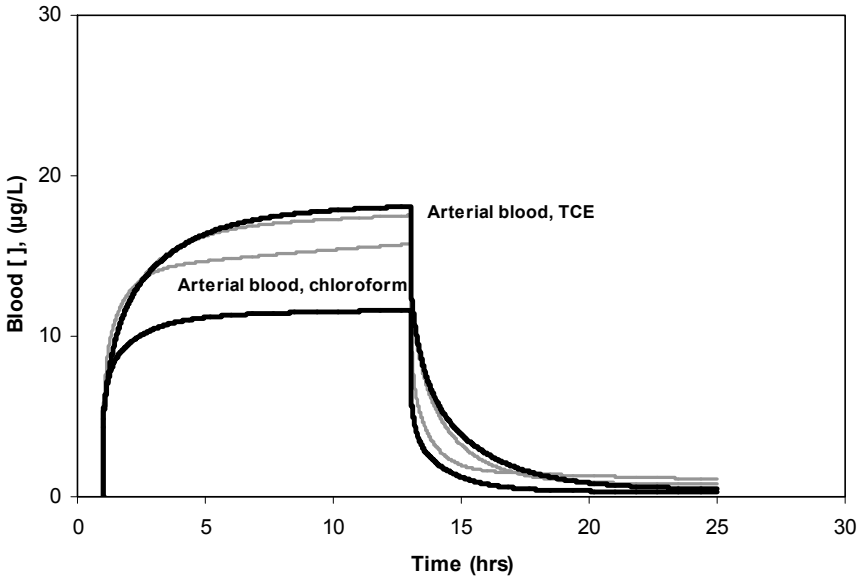
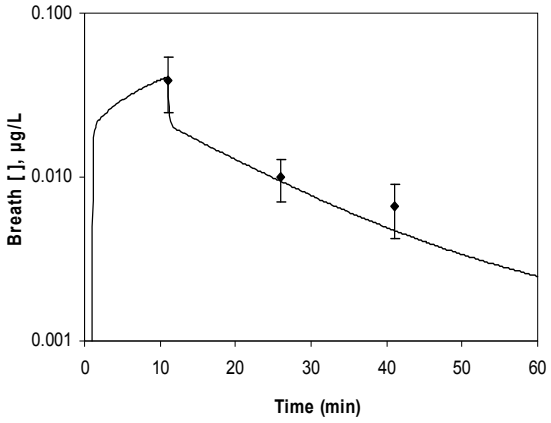
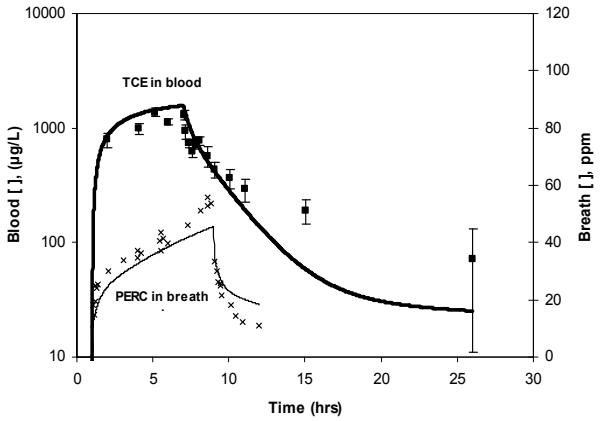

30
20
10
0
Blood [ ], (µg/L)
Arterial blood, TCE
Arterial blood, chloroform
0
5
10
15
20
25
30
Time (hrs)

Figure 2.3

a)



b)



c)

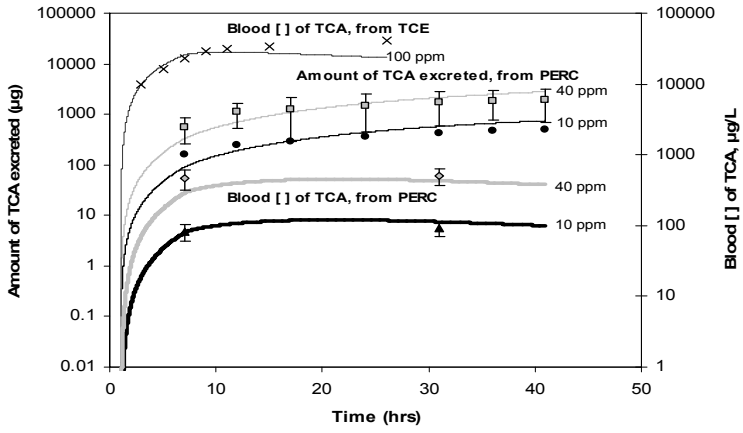
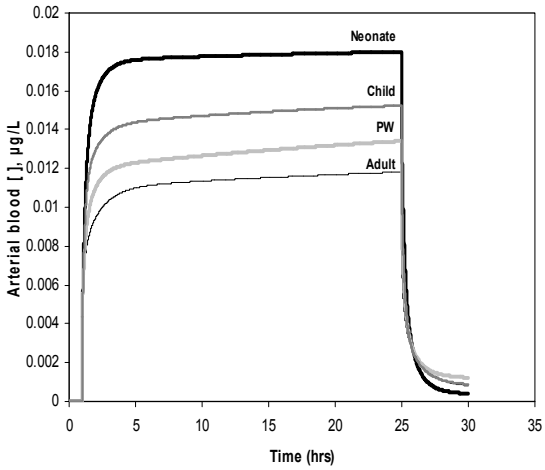
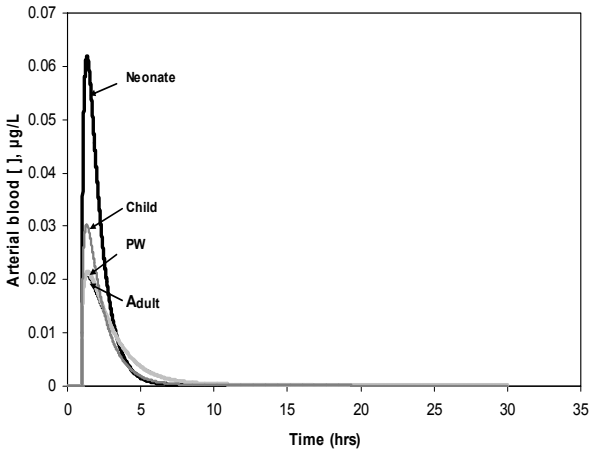


Figure 2.4

a)



b)



c)

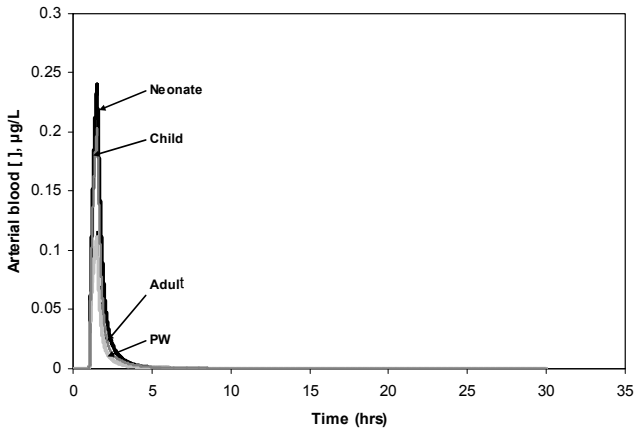


Table 2-I : Chemical-specific parameters for PBPK modelling

Parameters	Substances			
	Chloroform[a)]	Bromoform[a)]	TCE[a)]	PERC[b)]
Molecular weight (g/mol)[c)]	119.38	252.73	131.2	165.8
Absorption constants				
Oral ($min^{-1}/kg^{-0.25}$)	0.032	0.023	0.1667	0.00216[d)]
Dermal (cm/min)	0.00267	0.0035	0.002	0.00207[e)]
Urinary excretion constant of TCA ($min^{-1}/kg^{-0.25}$)	-	-	0.0012[f)]	0.0012[f)]
Partition coefficients				
Blood:air	7.43[g)]	102.3	9.2	11.58
Liver:air	17	210.3	62.56	61.14
Fat:air	280	4129	671.6	1449.8
Highly perfused tissues:air	17	210.3	62.56	58.7
Rest of the body:air	12	115.1	21.16	70.6
Skin:air	12[g)]	238.23	20.26	275.2[e)]
Kidney:air	11[g)]	173.4[h)]	-	58.7
Water:air	3.66	24.71	0.83	0.79[i)]
Placenta:blood	2.2[j)]	1.9[j)]	2.7[j)]	3.2[j)]
Metabolic constants				
Maximal rate ($\mu g/min\text{-}kg^{0.75}$)	211.33[g)]	173.33	166.67[k)]	4
Vmaxc proportionality constant kidney/liver	0.033[g)]	-	-	0.1
Michaelis-Menten (µg/L)	448[g)]	420	1500[k)]	7700
Fraction of metabolism resulting in TCA formation	-	-	0.25[l)]	0.6
Volume of distribution of TCA[m)]			$0.1 \times BW$	$0.1 \times BW$

Notes: a) Unless specified otherwise, values indicated are from Haddad *et al.* (2006). b) Unless specified otherwise, values indicated are from Clewell *et al.* (2005), based on Gearhart *et al.* (1993). c) ATSDR, 1997a-c, 2005. d) Human BW-adjusted value was obtained by scaling as a function of body surface from mouse duodenum data (0.005 min^{-1}) reported by Clewell *et al.* (2005) e) Rao and Brown, 1993. f) Mean of the adult values by Clewell *et al.* (2000) and Gearhart *et al.* (1993). g) Corley *et al.*, 1990. h) Tan *et al.*, 2007. i) Gargas *et al.*, 1989. j) Calculated according to Poulin and Krishnan (1995), based on composition of placenta described by Klingler *et al.* (2003). k) Clewell *et al.*, 2000. l) Based on data from Soucek *et al.* (1960), described in Clewell *et al.* (2000), m) Clewell *et al.* (2005) (based on Gearhart *et al.* (1993)).

Table 2-II : Description of the probabilistic and deterministic parameters in the PBPK models for each subpopulation evaluated

Parameter	Subpopulation Median age (range)	Adults 41 (18–64)	Neonates 14 d (0–30 d)	Children 2 (1–3)	Elderly 78 (65–90)	Pregnant women 29 (15–44)
PROBABILISTIC VARIABLES[a]						
Body weight						
(Kg, mean ± SD, range):[b]		76 ± 17, 37–152[c]	4 ± 1, 2–7[d]	13 ± 2, 7–32[c]	72 ± 16, 33–155[c]	82 ± 18, 48–166[e]
Body height						
(cm, mean ± SD, range):[b]		167 ± 10, 144–198[c]	46 ± 16, 35–80[f]	87 ± 6, 70–106[c]	164 ± 10, 138–190[c]	161 ± 7, 132–182[c]
CYP2E1 concentration						
(pmol/mg MSP, m ± SD):		49 ± 2, 11–130 [g]	18 ± 14, 1–56[d]	42 ± 18, 18–74[d]	h)	h)
DETERMINISTIC VARIABLE						
Glomerular filtration rate)						
(ml/min-1.73 m^2)		116.0[i]	40.2[i]	127.0[i]	92.4[i]	181.4[j]
VARIABILITY TERM (VT) [a]						
Cardiac output (Qc)		1 ± 0.09 (0.77–1.23)[k]	h)	h)	h)	h)
Alveolar ventilation rate (Qp)		1 ± 0.1 (0.8–1.2)[l]	h)	h)	h)	h)
Liver volume (Vl)		1 ± 0.14 (0.66–1.34)[m]	h)	h)	h)	h)
Liver blood flow (Ql)		1 ± 0.13 (0.67–1.33)[n]	h)	h)	h)	h)
Fat blood flow (Qf)		1 ± 0.07 (0.86–1.14)[l]	h)	h)	h)	h)

Notes a) Log normal distributions for probabilistic variables, normal distributions for VT. b) Body weights and heights are correlated at 60 %, based on body mass index distribution data for an adult population (Statistics Canada, 2003). c) P^3M Database. d) Johnsrud *et al.*, 2003. MSP: microsomal protein. e) Distribution from P^3M database, to which the mean body weight increase during pregnancy (12.5 kg), reported by ICRP (2002), was added. f) Nelson's Textbook of pediatrics (1991). g) Lipscomb *et al.* (2003) (Geometric mean ± geometric standard deviation. MSP: microsomal protein). h) Same as for adults. i) DeWoskin and Thompson (2008). In adults and elderly, based on a mean decrease of GFR of 0.67 % per year after age 30 from maximum value of 127 ml/min-1.73 m^2 (Sarangapani *et al.*, 2003). j) Based on a 30 % increase of GFR during pregnancy (Faustman and Ribeiro, 1990). k) Tan *et al.* (2007). l) Arbitrary. m) Thomas *et al.* (1996). n) Taniguchi *et al.* (1996).

Table 2-III : Values for physiological parameters for an average individual of each subpopulation evaluated, based on the equations described in Appendix B and mean values indicated in Table 2.II

Subpopulation Parameter	Adult[a)]	Neonate[b)]	Children[b)]	Elderly[a)]	Pregnant woman[c)]
Body surface area (cm^2)	18,299; 16,691	2,335	5,689	18,299; 16,691	18,662
Tissue volumes (% of body weight)					
Liver	2.6	3.7	2.9	2.6	2.2
Kidneys	0.4	0.7	0.5	0.4	0.4
Highly perfused tissues	7.0; 7.7	23.9; 21.1	15.4; 15.2	6.6; 7.9	12[d)]
Skin	5.0; 5.3	4.6	3.3	5.0; 5.3	4.3
Fat[e)]	20.0; 33.7	14.0	23.7; 26.5	20.0; 33.7	42.9[f)]
Bones	9.0	14.0; 15.7	14.5; 11.4	7.1; 8.3	7.6
Rest of the body	56.0; 41.2	39.1; 40.2	39.6; 40.1	58.2; 41.8	30.5
Cardiac output, Alveolar ventilation rate, (L/h)	383.1; 369.0	41.4; 42.0	103.8; 109.2	383.1; 369.0	559.8[g)]
Tissue blood flows (% of cardiac output)					
Liver	23.9; 25.4	17.1; 19.9	17.5; 19.9	23.9; 25.4	19.5
Kidneys	19.0; 17.0	13.5; 11.5	14.3; 11.9	19.0; 17.0	17.0
Highly perfused tissues	29.0; 28.5	57.8; 56.3	53.8; 52.6	29.1; 28.5	34.8[e)]
Skin	6.6; 7.8	3.0; 3.7	2.9; 3.5	6.6; 7.8	10.0
Fat[e)]	4.6; 6.9	1.6	3.6; 3.8	4.6; 6.9	7.9
Rest of the body	16.9; 14.5	7.0	7.9; 8.3	16.8; 14.5	10.8

Notes a) Double values denote values for male (BW = 70 kg, BH = 170 cm) and female (BW = 60 kg, BH = 165 cm female), for a 30 year old adult or a 78 year old elderly. b) Double values denote values for male and female, respectively, using age, BW and BH indicated in Table 2-II. c) Values calculated using the body weight of non-pregnant women (Table 2-II minus 12.5 kg), except body surface area as well as kidney's volume and blood flow. d) Includes foeto-placental unit. e) For PERC, compartment divided into two sharing 76.2 and 23.8 % of total fat volume and 60 and 40 % of total fat blood flow, as per Gearhart *et al.* (1993). f) Based on equations in Appendix B and a mean fat weight gain of 3.825 kg during pregnancy (ICRP, 2002). g) Based on a 34.5 % mean increase of cardiac output at 38th week of pregnancy (ICRP, 2002).

Table 2-IV : Human Kinetic Adjustment Factors (HKAF) obtained for inhalation exposure scenario in each subpopulation

Substance	**Chloroform**		**Bromoform**		**Trichloroethylene**			**Perchloroethylene**		
Dose surrogate	24-h AUC_{pc}	24-h AMET	24-h AUC_{pc}	24-h AMET	24-h AUC_{pc}	24-h AUC_{met}	24-h AMET	24-h AUC_{pc}	24-h AUC_{met}	24-h AMET
Adults										
Median	15.8	12.2	25.7	19.2	21.8	436	11.6	37.3	19.2	0.23
95th percentile	20.2	15.5	37.5	26.9	28.8	569	15.2	47.2	40.7	0.48
HKAF	**1.3**	**1.3**	**1.5**	**1.4**	**1.3**	**1.3**	**1.3**	**1.3**	**2.1**	**2.1**
Neonates										
95th percentile	33.4	18.7	93.1	38.1	48.4	897	15.8	66.6	39.9	0.32
HKAF	**2.1**	**1.5**	**3.6**	**2.0**	**2.2**	**2.1**	**1.4**	**1.8**	**2.1**	**1.4**
Children										
95th percentile	25.2	19.2	51.7	36.5	35.1	661	17.7	58.8	37.9	0.43
HKAF	**1.6**	**1.6**	**2.1**	**1.9**	**1.6**	**1.5**	**1.5**	**1.6**	**2.0**	**1.9**
Elderly										
95th percentile	20.4	15.6	37.6	26.8	28.8	601	15.3	45.8	42.9	0.48
HKAF	**1.3**	**1.3**	**1.5**	**1.4**	**1.3**	**1.4**	**1.3**	**1.2**	**2.2**	**2.1**
Pregnant women										
95th percentile	22.9	19.1	44.4	34.6	30.6	529	18.0	46.4	34.1	0.51
HKAF	**1.5**	**1.6**	**1.7**	**1.8**	**1.4**	**1.2**	**1.6**	**1.3**	**1.8**	**2.2**

Abbreviations: AMET, amount metabolized during 24 hours, normalized to liver volume (µg/24 h-L of liver); AUC, area under the arterial blood concentration vs time curve (µg.24 h/L); met, circulating metabolite; pc, parent compound. Shaded areas indicate values > 3.16; underlined values indicate subgroup with greater HKAF for corresponding internal dose surrogate (per column).

Table 2-V : Human Kinetic Adjustment Factors (HKAF) obtained for BW-adjusted oral exposure scenario in each subpopulation

Substance	**Chloroform**		**Bromoform**		**Trichloroethylene**			**Perchloroethylene**		
Dose surrogate	24-h AUC_{pc}	24-h AMET	24-h AUC_{pc}	24-h AMET	24-h AUC_{pc}	24-h AUC_{met}	24-h AMET	24-h AUC_{pc}	24-h AUC_{met}	24-h AMET
Adults										
Median	3.4	35.7	6.3	37.6	13.7	2104	30.1	38.0	41.3	0.42
95th percentile	7.0	45.5	13.5	47.6	24.7	2380	38.9	48.1	83.0	0.85
HKAF	**2.1**	**1.3**	**2.1**	**1.3**	**1.8**	**1.1**	**1.3**	**1.3**	**2.0**	**2.0**
Neonates										
95th percentile	16.3	28.0	46.9	32.3	34.9	2094	21.3	53.2	59.5	0.37
HKAF	**4.9**	**0.8**	**7.4**	**0.9**	**2.6**	**1.0**	**0.7**	**1.4**	**1.4**	**0.9**
Children										
95th percentile	6.3	39.8	13.5	42.7	21.5	1847	32.8	53.2	66.3	0.63
HKAF	**1.9**	**1.1**	**2.1**	**1.1**	**1.6**	**0.9**	**1.1**	**1.4**	**1.6**	**1.5**
Elderly										
95th percentile	7.3	45.6	13.5	46.9	25.0	2584	38.7	49.3	90.0	0.86
HKAF	**2.2**	**1.3**	**2.1**	**1.3**	**1.8**	**1.2**	**1.3**	**1.3**	**2.2**	**2.1**
Pregnant women										
95th percentile	7.5	53.3	14.6	56.3	23.7	2095	44.2	39.4	62.6	0.81
HKAF	**2.3**	**1.5**	**2.3**	**1.5**	**1.7**	**1.0**	**1.5**	**1.0**	**1.5**	**1.9**

Abbreviations: AMET, amount metabolized during 24 hours, normalized to liver volume (µg/24 h-L of liver); AUC, area under the arterial blood concentration vs time curve (µg.24 h/L); met, circulating metabolite; pc, parent compound. Shaded areas indicate values > 3.16; underlined values indicate subgroup with greater HKAF for corresponding internal dose surrogate (per column).

Table 2-VI : Human Kinetic Adjustment Factors (HKAF) obtained for dermal exposure scenario in each subpopulation

Substance	**Chloroform**		**Bromoform**		**Trichloroethylene**			**Perchloroethylene**		
Dose surrogate	24-h AUC_{pc}	24-h AMET	24-h AUC_{pc}	24-h AMET	24-h AUC_{pc}	24-h AUC_{met}	24-h AMET	24-h AUC_{pc}	24-h AUC_{met}	24-h AMET
Adults										
Median	5.5	4.2	12.1	9.1	5.8	207	3.1	11.7	9.2	0.07
95th percentile	7.3	5.4	17.6	12.4	8.3	278	4.2	16.8	19.4	0.16
HKAF	**1.3**	**1.3**	**1.5**	**1.4**	**1.4**	**1.3**	**1.4**	**1.4**	**2.1**	**2.3**
Neonates										
95th percentile	15.1	8.0	53.7	21.7	18.2	562	5.6	30.6	26.2	0.13
HKAF	**2.8**	**1.9**	**4.4**	**2.4**	**3.1**	**2.7**	**1.8**	**2.6**	**2.9**	**1.9**
Children										
95th percentile	10.1	7.6	28.2	19.3	11.8	350	5.7	24.3	22.6	0.17
HKAF	**1.9**	**1.8**	**2.3**	**2.1**	**2.0**	**1.7**	**1.8**	**2.1**	**2.5**	**2.4**
Elderly										
95th percentile	7.3	5.4	18.3	12.5	8.3	296	4.3	16.9	21.0	0.16
HKAF	**1.3**	**1.3**	**1.5**	**1.4**	**1.4**	**1.4**	**1.4**	**1.5**	**2.3**	**2.3**
Pregnant women										
95th percentile	6.7	5.6	16.7	13.2	7.0	190	4.0	12.8	12.5	0.19
HKAF	**1.2**	**1.3**	**1.4**	**1.5**	**1.2**	**0.9**	**1.3**	**1.1**	**1.4**	**1.9**

Abbreviations: AMET, amount metabolized during 24 hours, normalized to liver volume (µg/24 h-L of liver); AUC, area under the arterial blood concentration vs time curve (µg.24 h/L); met, circulating metabolite; pc, parent compound. Shaded areas indicate values > 3.16; underlined values indicate subgroup with greater HKAF for corresponding internal dose surrogate (per column).

Appendix A: Values of K_u for TCA in various subpopulations, on the basis of the mean volume-adjusted renal blood flow (Qk_{ad}) and the renal extraction ratio (E_{ren})

	BW	Vk	Qk		GFR		E_{ren}	(Qk_{ad} x E_{ren})	Ratio vs	K_u used in PBPK models
	g	ml	ml/min	ml/min.g of k	ml/min.1.73m^2	ml/min		ml/min.g of k	adult	min^{-1}/kg$^{-0.25\ a)}$
Adult	75,400	287	1288	4.49	116	127	0.09	0.40	1.000	0.0012
Neonate	3750	25	93	3.72	40	5.4	0.05	0.20	0.507	0.0006
Child	12,600	67	248	3.70	127	42	0.14	0.54	1.331	0.0015
Elderly	71,600	275	1237	4.50	92	97	0.07	0.33	0.812	0.0009
PW	82,440	308	1587	5.15	181	196	0.11	0.57	1.406	0.0016

a): Calculated as the ratio *vs* adult x 0.0012 (Gearhart et al. (1993) and Clewell et al. (2000), see Table 1)

Appendix B: Calculation of the physiological parameters in each subgroup

Parameter / **Subpopulation**	**Equation**	**Reference**
- Body surface area (SA, cm^2)		
ad, neo, child, eld	$= BW^{0.515} \times BH^{0.422} \times 234.9$	Haddad *et al.*, 2006
PW	$= BW^{0.425} \times BH^{0.725} \times 71.84$	Wang *et al.*, 1992
- Tissue volumes (L)		
Liver (Vl)		
ad, eld, PW	$= 0.026 \times BW$	Brown *et al.*, 1997
neo, child	$= 0.05012 \times BW^{0.78}$	Haddad *et al.*, 2006
Kidney (Vk)		
All	$= ((4.214 \times BW^{0.823}) + (4{,}456 \times BW^{0.795})) / 1000$	Price *et al.*, 2003b
Highly perfused (Vh)[a)]		
ad, eld, PW		Haddad *et al.*, 2006
♂	$= -5.309E\text{-}3 \times age + 1.008 \times BW^{0.493} + BH^{0.3765} - 7.952$	
♀	$= -2.331E\text{-}3 \times age + 0.1253 \times BW^{0.8477} + BH^{0.3821} - 4.725$	
neo, child		
♂	$= -1.0682E\text{-}2 \times age + 2.038 \times (BW^2 / BH)^{0.4014} - 0.2046$	
♀	$= -1.919E\text{-}2 \times age + 3.193 \times (BW^2 / BH)^{0.2657} - 1.374$	
Skin (Vs)		
ad, eld, PW	$= (1.834 \times (SA / 10^4)) + (7.85E\text{-}2 \times (SA / 10^4)^{1.049})$	Haddad *et al.*, 2006
neo, child	$= (0.664 \times (SA / 10^4)) + (7.85E\text{-}2 \times (SA / 10^4)^{1.049})$	
Fat (Vf)		
ad, eld		
♂	$= [1.36 \times BW / (BH / 100)] - 42$	Price *et al.*, 2003b
♀	$= [1.61 \times BW / (BH / 100)] - 38.3$	

child		Price *et al.*, 2003b
♂	= (908.4 + 0.706 × (BW × 1000) - 53 × BH - 3.057 × (age × 365.25)) / 1000	
♀	= (908.4 + 0.706 × (BW × 1000) - 53 × BH + 358.5 - 3.057 × (age × 365.25)) / 1000	
neo[b)]	= 0.14 × BW	Haddad *et al.*, 2001
PW[c)]	[1.61 × BW / (BH / 100)]- 38.3 + 3.825	Price *et al.*, 2003b; ICRP, 2002
Bone (Vb)		
ad, PW[d)]	= 0.09 × BW	Tan *et al.*, 2007
eld[e)]		
♂	= 0.09 × 46/58 × BW	Tan *et al.*, 2007; Price *et al.*, 2003b Haddad *et al.*, 2001
♀	= 0.09 × 47/51 × BW	
neo, child[f)]		
♂	= [(-0.0306 × age^5 + 0.5222 × age^4 + 9.7109 × age^3-187.97 × age^2 + 1089.7 × age + 546.6) / 1000] - [(0.0019956 × age^6 - 0.11169 × age^5 + 2.189 × age^4 -17.726 × age^3 + 59.767 × age^2 + 14.405 × age + 73.716) / 1000]	
♀	= [(-0.002831 × age^5 + 0.18184 × age^4 + 10.685 × age^3-142.88 × age^2 + 782.05 × age + 609.64) / 1000] - [(0.0007984 × age^6 - 0.037966 × age^5 + 0.5272 × age^4 - 1.1311 × age^3 -12.285 × age^2 + 123.87 × age + 53.358) / 1000]	
Foeto-placental unit (Vpl)	= 0.0618 × BW_{PW}	ICRP, 2002
foetus only	= 0.677 × Vpl	ICRP, 2002
Rest of the body (Vr)		
ad, neo, child, eld	= BW - (Vb + Vf + Vs + Vh + Vk + Vl)	
PW	= BW - (Vpl + Vb + Vf + Vs + Vh + Vk + Vl)	
<u>- Flows (L/min)</u>		
Cardiac output (Qc)		
Ad, neo, child, eld		Haddad *et al.*, 2006
♂	= 0.2519 × BW$^{0.7609}$	

♀	$= 0.2508 \times BW^{0.7815}$	Haddad *et al.*, 2006; ICRP, 2002
PW[g)]	$= (0.2508 \times BW^{0.7815}) \times 1.346$	
Alveolar ventilation (Qalv)		
all	= Qc	Haddad *et al.*, 2006
Liver (Ql)		
all		Haddad *et al.*, 2006
♂	$= 0.84 \times Vl$	
♀	$= 1.00 \times Vl$	
Kidney (Qk)		
ad, eld, PW		Brown *et al.*, 1997
♂	$= 0.19 \times CO$	
♀	$= 0.17 \times CO$	
neo, child		Price *et al.*, 2003b
♂	$= 3.68 \times Vk$	
♀	$= 3.22 \times Vk$	
Skin (Qs)		
Ad, neo, child, eld		Haddad *et al.*, 2006
♂	$= 0.12 \times Vs$	
♀	$= 0.13 \times Vs$	
PW	$= 1.74 \times (0.13 \times Vs)$	Haddad *et al.*, 2006; Faustman and Ribeiro, 1990
Fat (Qf)		Haddad *et al.*, 2006
♂	$= 0.0209 \times Vf$	
♀	$= 0.03 \times Vf$	
Foeto-placental unit (Qpl)		
foetus only	$= 0.12 \times Qc$	ICRP, 2002

	= 0.017	Clewell *et al.*, 1999
Rest of the body (Qr)[h)]		
ad, eld, PW		Haddad *et al.*, 2006
♂	$= 1.1 \times [(0.03 \times ((1.19E\text{-}3 \times BW - 4.302\ E\text{-}4) + (2.598E\text{-}1 \times BW + 1.206E\text{-}1 \times BH - 4.3E\text{-}3 \times age - 1.11)) + (0.73 \times 1.017\ E\text{-}7 \times (BH^{0.664} \times BW^{0.3851} \times 242.7)^{1.42})]$	
♀	$= 1.1 \times [(0.03 \times ((1.19E\text{-}3 \times BW - 4.302\ E\text{-}4) + (6.78 \times (SA/10^4)^{1.69} - 1.492E\text{-}3 \times age + 3.58)) + (0.96 \times 1.017\ E\text{-}7 \times (BH^{0.6862} \times BW^{0.3561} \times 242.7)^{1.42})]$	
neo, child		
♂	$= 1.1 \times [(0.03 \times ((1.19E\text{-}3 \times BW - 4.302\ E\text{-}4) + (9.561\ E\text{-}2 \times BW + 1.601E\text{-}2 \times BH + 1.097E\text{-}1 \times age)) + (0.73 \times 1.017\ E\text{-}7 \times (BH^{0.664} \times BW^{0.3851} \times 242.7)^{1.42})]$	
♀	$= 1.1 \times [(0.03 \times ((1.19E\text{-}3 \times BW - 4.302\ E\text{-}4) + (9.563E\text{-}2 \times BW + 1.65E\text{-}2 \times BH + 9.102E\text{-}2 \times age - 0.1642)) + (0.96 \times 1.017\ E\text{-}7 \times (BH^{0.6862} \times BW^{0.3561} \times 242.7)^{1.42})]$	
Highly perfused (Qh)		
ad, neo, child, eld	= CO - (Qf + Qs + Qr + Qk + Ql)	
PW	= CO - (Qpl + Qf + Qs + Qr + Qk + Ql)	

a): Volumes of liver and kidney (when considered) are substracted from the result of this equation in order to obtain volume of the highly perfused tissue in the PBPK models.

b) Data presented by Haddad *et al.* (2001) show that adipose tissue represent roughly 14 % of neonates BW born at term.

c) Equation of Price *et al.* (2003b), for non-pregnant women, to which the mean fat weight gain during pregnancy, as reported by ICRP (2002), was added.

d) For pregnant women, the volume of the bone was based on the BW of a non-pregnant woman.

e) 46/58 and 47/51 represent the ratio of the proportion of total body bone mineral on lean body mass between elderly and young adults in males and females, respectively, as reported by Price *et al.* (2003b).

f) The volume of the bones was computed as the difference between the total volume of bone plus marrow and the volume of marrow alone, both of which were calculated as per Haddad *et al.* (2001)

g) The cardiac output of non-pregnant women was increased by a factor of 1.346 based on the mean increase of cardiac output at the 38th week of pregnancy (ICRP 2002).

Appendix C: Mean internal dose surrogates values obtained for each subgroups and exposure scenario

Substance	**Chloroform**		**Bromoform**		**Trichloroethylene**			**Perchloroethylene**		
Dose surrogate	24-h AUC_{pc}	24-h AMET	24-h AUC_{pc}	24-h AMET	24-h AUC_{pc}	24-h AUC_{met}	24-h AMET	24-h AUC_{pc}	24-h AUC_{met}	24-h AMET
Subpopulation Scenario										
Adults										
24-h Inhalation	15.9	12.4	26.4	19.8	22.1	439	11.7	37.8	21.2	0.25
Oral, 1 µg/kg-d	3.7	36.3	7.0	38.1	14.6	2076	30.5	38.5	44.9	0.46
30 min Dermal	5.6	4.3	12.5	9.3	6.0	210	3.2	12.0	10.1	0.08
Neonates										
24-h Inhalation	26.4	14.6	58.1	29.6	39.5	576	10.5	62.5	17.9	0.14
Oral, 1 µg/kg-d	7.9	20.8	20.0	25.4	22.2	1409	13.9	45.0	25.2	0.15
30 min Dermal	11.3	6.2	33.9	17.2	13.8	361	3.7	25.0	11.0	0.05
Children										
24-h Inhalation	21.1	15.7	38.7	28.1	29.4	512	14.0	52.2	22.6	0.27
Oral, 1 µg/kg-d	3.8	32.1	8.0	34.3	14.7	1616	25.8	45.1	40.2	0.39
30 min Dermal	8.4	6.3	21.3	15.5	9.5	275	4.5	19.4	13.0	0.10
Elderly										
24-h Inhalation	16.1	12.5	26.4	19.9	22.2	457	11.6	37.3	22.2	0.25
Oral, 1 µg/kg-d	3.8	36.3	7.1	38.0	14.8	2248	30.4	38.6	47.8	0.46
30 min Dermal	5.6	4.3	12.6	9.5	6.1	227	3.2	12.2	10.9	0.08
Pregnant women										
24-h Inhalation	18.6	15.5	32.3	26.3	24.9	407	13.9	39.7	16.8	0.27
Oral, 1 µg/kg-d	3.9	42.5	7.6	4.4	14.4	1792	34.3	32.9	33.5	0.43
30 min Dermal	5.0	4.1	11.7	9.5	5.2	144	2.9	9.4	6.6	0.10

3 Article II : *Assessing the impact of the duration and intensitiy of inhalation exposure on the magnitude of the variability of internal dose metrics in children and adults*

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ASSESSING THE IMPACT OF THE DURATION AND INTENSITIY OF INHALATION EXPOSURE ON THE MAGNITUDE OF THE VARIABILITY OF INTERNAL DOSE METRICS IN CHILDREN AND ADULTS

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Abstract

The objective of this study was to assess the impact of the exposure duration and intensity on the human kinetic adjustment factor (HKAF). A physiologically-based pharmacokinetic model was used to compute targeted dose metrics (*i.e.* maximum blood concentration (Cmax) and amount metabolized/L liver/24-h (Amet)) in adults, neonates (0–30 d), toddlers (1–3 yr) and pregnant women following inhalation exposure to benzene, styrene, 1,1,1-trichloroethane and 1,4-dioxane. Exposure scenarios simulated involved various concentrations based on the chemical's RfC (low) and six of U.S. EPA's Acute Exposure Guideline Levels (AEGLs) (high), for durations of 10 min, 60 min, 8 h and 24 h, as well as at steady-state. Distributions for body weight (BW), height (H) and hepatic CYP2E1 content were obtained from the literature or from P3M software whereas blood flows and tissue volumes were calculated from BW and H. The HKAF was computed based on distributions of dose metrics obtained by Monte Carlo simulations as [95^{th} percentile in each subpopulation/median in adults]. At low levels of exposure, ranges of Cmax-based HKAF were 1–6.8 depending on the chemical, with 1,4-dioxane exhibiting the greatest values. At high levels of exposure, this range was 1.1–5.2, with styrene exhibiting the greatest value. Neonates were always the most sensitive subpopulation based on Cmax, and pregnant women based on Amet in the majority of the cases (1.3–2.1). These results have shown that the chemical-specific HKAF varies as a function of exposure duration and intensity of inhalation exposures, and sometimes exceeds the default values used in risk assessments.

Keywords: AEGL, Human kinetic adjustment factor, Interindividual variability, Physiologically-based pharmacokinetics (PBPK), Risk assessment, Toxicokinetics, VOCs

LIST OF ABBREVIATIONS AND ACRONYMS

AEGL	**acute exposure guideline**
Amet	**amount metabolized**
AUC	**area under blood concentration *vs* time curve of the parent compound**
Cmax	**maximum blood concentration of parent compound**
CNS	**central nervous system**
CSAF	**chemical-specific adjustment factor**
CYP2E1	**cytochrome P-450 2E1**
HEAEGL	**human equivalent acute exposure guideline**
HEC	**human equivalent concentration**
HKAF	**human kinetic adjustment factor**
Km	**Michaëlis-Menten constant**
Pb	**blood:air partition coefficient**
PBPK	**physiologically-based pharmacokinetic**
RAM	**rate of metabolism**
RfC	**reference concentration**
TK	**toxicokinetics**
TRI	**1,1,1-trichloroethane**
UF	**uncertainty factor**
Vmax	**maximum rate of metabolism**
VOC	**volatile organic compound**

3.1 Introduction

A default uncertainty factor (UF) of 10 is generally applied to account for the human interindividual variability in non-cancer risk assessment (Dourson *et al.*, 1996; Dourson and Stara, 1983; U.S. EPA, 2002). Besides, UFs of different magnitude, between 1 and 10 depending on the chemical, are used by the U.S. EPA to establish the Acute Exposure Guideline Levels (AEGLs) for various chemicals (NRC 2001). These guidelines include recommended maximum airborne concentrations of various chemicals for different exposure durations (10 min - 8 h). They are established to protect the most susceptible individuals of the population from selected acute adverse health effects, namely:

- asymptomatic or transient and reversible effects of discomfort and irritation (AEGL-1);

- irreversible or serious, long-lasting adverse health effects or an impaired ability to escape (AEGL-2); and

- life-threatening effects (AEGL-3).

Conceptually, the interindividual variability in toxicokinetics (TK) and toxicodynamics (TD) can be considered as separate contributors to the overall variability underlying the default interindividual UF of 10. Thus, both of these components can be given a default value of √10, or 3.2 (Dorne and Renwick 2005; IPCS, 1994; Renwick and Lazarus 1998). This default value for chemicals can be assessed and replaced as appropriate by quantifying the data-derived chemical-specific adjustment factors (CSAFs), as proposed by the International Programme on Chemical Safety (Clewell *et al.*, 2008; IPCS, 2005; Meek *et al.*, 2002). Using this approach, the CSAF for interindividual variability in TK, also referred to as the human kinetic adjustment factor (HKAF), can be determined using the population distributions of the relevant pharmacokinetic parameters (e.g., the half-life,

blood concentration), which reflect the population variability in the determinants of TK. The HKAF is computed as the ratio of an upper percentile value (e.g., the 95th or the 99th) of the parameter in a population to its central tendency (e.g., the median), or as the ratio of an upper percentile value in a sensitive subpopulation to its central tendency in average healthy individuals (IPCS, 2005; Meek *et al.*, 2002).

The magnitude of the HKAF for oral exposure to chemicals has been calculated using an analysis of the therapeutic drug database for metabolic pathway-related differences in pharmacokinetic parameters among healthy adults and other subpopulations (Dorne *et al.*, 2005; Ginsberg *et al.*, 2002). Because ethical considerations preclude obtaining data on environmental toxicants in sensitive subpopulations, physiologically-based pharmacokinetic (PBPK) models (Clewell *et al.*, 2004; Gentry *et al.*, 2002, Mörk and Johanson, 2010, Nong *et al.*, 2006; Pelekis *et al.*, 2001, 2003; Sarangapani *et al.*, 2003) and steady-state algorithms (Nong and Krishnan 2007; Pelekis *et al.* 2001) have been used to explore the adequacy of the HKAF with regard to these chemicals.

The large majority of the published modeling studies have focused on a single exposure scenario (often inhalation) in terms of its duration and intensity. However, few studies have reported that the magnitude of the average child-to-adult ratio of blood concentration of inhaled styrene and dichloromethane could vary as a function of the airborne concentration and exposure duration (Abraham *et al.*, 2005a; Mielke *et al.*, 2005). The results from Abraham *et al.* (2005b) also suggested that this ratio may vary with physico-chemical characteristics, but in-depth analysis of the impact of the various systemic clearance processes involved have not been undertaken to date. Besides, the impact of the exposure duration and intensity on the magnitude of the human TK variability have not been elucidated using a probabilistic approach consistent with the IPCS framework for quantifying HKAFs. Therefore, the objective of this study was to evaluate the impact of the

exposure intensity and duration on the magnitude of the HKAFs for several subpopulations *i.e.* neonates, adults, toddlers and pregnant women.

3.2 Methods

A physiologically-based pharmacokinetic (PBPK) model was applied to compute the HKAFs using probabilistic distributions of internal dose metrics of selected chemicals that exhibited a range of physico/biochemical properties. These distributions were obtained in adults and in several sensitive subpopulations using Monte Carlo simulations. The subpopulation-specific HKAF was then computed as the ratio of the 95th percentile of the DM in each subpopulation over the median in adults.

3.2.1 Selection of surrogate chemicals and their specific parameters

Four VOCs for which AEGLs have been determined by the U.S. EPA, generally based on acute systemic effects on the central nervous system (CNS) (U.S. EPA 2009b, 2008, 2005, 2000), were selected for the present study (Table 3-I). These VOCs were chosen according to the following criteria: the availability of a PBPK model and parameters in the literature, the variability of the pulmonary clearance potential (as defined by the range of the blood:air partition coefficient (Pb)), and the variability in the hepatic metabolism with regard to the extraction ratio. Benzene was chosen as a highly-metabolized VOC with a high level of pulmonary clearance (Pb = 7.4). Styrene was selected as a highly metabolized substrate with lower pulmonary clearance (Pb = 52). 1,1,1-trichloroethane (TRI) was chosen as surrogate for chemicals exhibiting lower hepatic clearance coupled with higher pulmonary clearance (Pb =2.53). Lastly, 1,4-dioxane was used as surrogate for chemicals with lower hepatic and pulmonary clearance (Pb = 3650). Three out of the four VOCs chosen for the present study are known substrates of cytochrome P-450 2E1 (CYP2E1) (Ronis et al,

1996), for which extensive data on the interindividual variability are available (Johnsrud et al., 2003; Lipscomb et al., 2003). Additionally 1,4-dioxane was included in this study to facilitate the coverage of a range of physico/biochemical properties of potential substrates of CYP2E1 (Nannelli et al., 2005).

3.2.2 PBPK model structure and parameters for specific subpopulations

A five-compartment PBPK model written in Microsoft Excel® (Microsoft Corporation, Seattle, WA) as per Haddad *et al.* (1996) was used. This simple model structure was deemed adequate given the dose metrics of interest for the present study (see below), and is similar to previously published models suitable for the study of inhalation kinetics (Fig. 3.1). Accordingly, chemical-specific parameters (Table 3-I) were taken from the literature (Haddad *et al.*, 2001a; Lu *et al.*, 2008; Ramsey and Andersen 1984; Reitz *et al.*, 1990), except for the placenta:blood partition coefficients, which were calculated using the placenta composition data (Klingler *et al.*, 2003; Poulin and Krishnan, 1995). Also, the fetus:blood partition coefficient was assumed to be equal to the value for the highly perfused tissues. For what concerns the tissue blood flows and volumes in the model that was used, they are determined based on the equations described by Price *et al.* (2003), revised by Haddad *et al.* (2006) and slightly modified afterwards (Valcke and Krishnan, 2011). These equations allow for the calculation of physiological parameters as a function of four determinants, *i.e.* body weight, height, age, and gender (Table 3-II), which result in the physiological parameters being correlated for a given set of values for these determinants. Additionally, the model includes variability terms, which are detailed in Valcke and Krishnan (2011), as multipliers of the calculated cardiac output, alveolar ventilation rate, fat blood flow, and liver volume/blood flow for a given set of body weight and height data. The use of variability terms contributes to account for the interindividual differences in the physiology of persons of identical age and body mass index. Generally, the parameter-specific equations of Table 3-II are the same as those used by Valcke and Krishnan (2011). However, these equations were averaged for both men and women

because the gender-related differences in the blood TK of these VOCs are likely to be negligible (Clewell *et al.*, 2004; Sarangapani *et al.*, 2003). Finally, the model allows for the consideration of the catalytic turnover of CYP2E1 (Valcke and Krishnan, 2011). For validation purposes, the mean age, body weight and height (when available) of volunteers participating in experimental trials with the studied chemicals (Table 3-I), along with experimental conditions, were entered as input data in the model, and the kinetics simulated was compared to experimental measurements.

Table 3-III shows the statistics of the physiological determinants of the investigated subpopulations. Subpopulation-specific data were obtained from the P^3M database (The Lifeline Group Inc, Annandale, VA; Price *et al.*, 2003) and the literature (Faustman and Ribeiro, 1990; ICRP, 2002; Nelson, 1991; Sarangapani *et al.*, 2003; Tan *et al.*, 2007; Taniguchi *et al.*, 1996; Thomas *et al.*, 1996). Adults were considered to be the reference subpopulation, whereas neonates (birth–30 d), toddlers (1–3 yr), and pregnant women in their 38^{th} week were presumed to be more sensitive pharmacokinetically. In other terms, greater dose metrics in a subpopulation compared to mean dose metrics in adults is meant to represent the pharmacokinetic basis of increased susceptibility of the particular subpopulation to the acute CNS effects underlying the AEGL determination of the investigated VOCs.

3.2.3 Exposure conditions and dose metrics retained

The subpopulation-specific PBPK models were used to simulate the inhalation kinetics of each substance following exposure to a range of concentrations and durations that were relevant to their AEGLs (Table 3-IV). The exposure concentrations were determined as human equivalent AEGL (*i.e.* HEAEGL) by multiplying the chosen AEGL value by the corresponding interindividual UF for each chemical (Table 3-IV). These values were within

the range of concentrations simulated elsewhere, *i.e.* 1–10,000 ppm (Abraham *et al.*, 2005b; Mielke *et al.*, 2005). For scenarios relevant to AEGL-1 and AEGL-2, exposure durations of 10 min, 1 h, and 8 h were considered. However, HEAEGL-1 was not simulated for 1,4-dioxane because the critical effect on which its AEGL-1 is based is local irritation rather than systemic CNS effects. For purpose of comparison with environmentally-relevant exposure, 8- and 24-h exposure, as well as steady-state conditions calculated as per Pelekis *et al.* (1997), were obtained for an exposure level corresponding to 10 × the reference concentration (referred to as "human equivalent concentration" (HEC)). Primary dose metrics considered was the maximum arterial blood concentration (Cmax) as it has been shown to be better correlated with acute CNS effects of VOCs (Benignus *et al.*, 1998, 2009; Boyes *et al.*, 2000). The 24-h amount metabolized normalized to liver volume (Amet) was also considered for comparison purpose.

3.2.4 Probabilistic modeling and calculation of HKAFs

For each subpopulation and exposure condition, Monte Carlo simulations were performed using Crystal Ball software (Oracle™, Redwood Shores, CA) to generate the distributions for relevant dose metrics after 2000 iterations. Based on the distribution of the body mass index in the Canadian adult population (Statistics Canada, 2003), a 60 % coefficient of correlation between the body weight and body height was applied to avoid unrealistic combinations at each Monte Carlo iteration. The dose metric distributions resulting from the Monte Carlo simulations were used to calculate the HKAF in each subpopulation as the ratio of their 95th percentile value over the median in adults (IPCS, 2005).

3.3 Results

3.3.1 Validation of the PBPK models

Fig. 3.2 shows that the PBPK model used in the present study predicted the kinetics of benzene and styrene well, as observed respectively by Pekari *et al.* (1992) and Ramsey *et al.* (1980). The fit was average with the kinetics of TRI and 1,4-dioxane as observed by Nolan *et al.* (1984) and Sweeney *et al.* (2008), respectively. But in the case of 1,4-dioxane, the fit obtained was reasonable only for Cmax, a DM relevant for this study. No attempt was made to optimize individual chemical PBPK model fits. The focus was to characterize the variability in DMs associated with a certain combination of metabolic and pulmonary clearance values.

3.3.2 Simulation of acute exposures

Fig. 3.3 shows the arterial blood concentrations of each chemical for an 8-h exposure to the HEC in average individuals for each subpopulation (Table 3-III). In every case, the neonate exhibited the highest peak blood concentrations. However, the blood concentrations of benzene, styrene, and TRI declined more rapidly in the neonates than in the adults (or pregnant women) when the exposure was stopped and were lower in the neonates than in the adults (or pregnant women) at the end of the simulation. Pregnant women always exhibited higher peak blood concentrations than the adult, but exhibited lower peak blood concentrations than the average toddler.

On the basis of mean body weight and hepatic CYP2E1 content (Table 3-III), Vmax values calculated for the average adult for benzene, styrene, TRI, and 1,4-dioxane were 786, 3111, 156, and 98 μg/min, respectively, whereas the corresponding values in the average neonate were 23, 88, 5, and 3 μg/min. Fig. 3.4 shows the kinetics of investigated chemicals at

HEAEGL-2 in the average adult and neonate. Saturation was reached for benzene (A), styrene (B) and 1,4-dioxane (D) as the rate of metabolism (RAM) = Vmax, but not for TRI as RAM never reached the Vmax (C). The RAM slowed down immediately when the exposure was stopped in the adult and neonate groups for benzene and TRI. In addition, the RAM of styrene slowed down immediately in the adult but remained near Vmax during the entire simulation in the neonate, equally as for 1,4-dioxane.. For benzene, styrene and 1,4-dioxane, lower exposure levels did not yield saturation of the metabolism (not shown).

Finally, Fig. 3.5 shows the kinetics of benzene and styrene at HEAEGL-1 in neonates and adults. At the end of the 8-h exposure to benzene (A), metabolism has reached approximately 36 % of saturation in adults, based on the value of Vmax, *i.e.* 285 µg/min *vs* 786 µg/min. In neonates, the saturation was 70 % (16 µg/min *vs* 23 µg/min). In the case of styrene (B), the corresponding numbers are 13 % in adults (416 µg / min *vs* 3111 µg/min) and 48 % in neonates (40 µg/min *vs* 83 µg/min).

3.3.3 HKAF for different durations

The chemical-specific HKAFs for each subpopulation and exposure condition are shown in Table 3-V, whereas the median dose metrics, including 24-h area under the blood concentration *vs* time curve (AUC), are reported in the Appendix. Considering the parent compound as the toxic moiety of interest, the neonates constituted the most sensitive subpopulation, as they always present the highest HKAF values based on Cmax. This finding is linked to the increased intake as compared to other subpopulations because of a greater body weight-adjusted alveolar ventilation rate (Mielke *et al.*, 2005; Valcke and Krishnan, 2009) combined with a reduced metabolic capacity of CYP2E1 (Table 3-III). Based on Amet, pregnant women constituted the most sensitive subpopulation, dosimetrically speaking, in two thirds of the cases and the second most sensitive in the other third. High Amet-based HKAF in pregnant women is likely to have resulted from

high BW-adjusted intakes of the parent compound (Faustman and Ribeiro, 1990; Valcke and Krishnan, 2009) combined with efficient metabolism.

For the remainder of the results section, the term "HKAF" will refer to the highest toxicokinetic interindividual variability factor obtained among those for all subpopulations studied and these are indicated in bold in Table 3-V. The greatest Cmax-based HKAFs were obtained for 1,4-dioxane for the low exposure level (3.1–6.8) and for the high exposure levels over 10–60 min (2.2–2.6). For an 8-h high level of exposure, the greatest Cmax-based HKAFs were obtained for styrene (3.4 and 5.2). TRI exhibited the greatest, but slightly variable, Amet-based HKAFs, with values ranging from 2.0 to 2.1. Actually, no significant trends could be detected with regard to Amet-based HKAFs for the various scenarios simulated. Cmax-based HKAFs obtained for TRI were the lowest among the investigated chemicals, with the greatest value being 1.3 for this poorly metabolized chemical exhibiting a high pulmonary clearance. For benzene, that is extensively cleared by both pulmonary and hepatic clearances, the Cmax-based HKAFs for various exposures duration/concentrations were comparable (1.5 to 2.7). For styrene and 1,4-dioxane, for which the pulmonary clearance is reduced, the difference among the HKAFs for various exposure scenarios/concentrations is more significant. The Cmax-based HKAF varied from 1.4 to 5.2 for styrene and from 2.2 to 4.0 for 1,4-dioxane.

The exposure conditions also influenced the Cmax-based HKAFs for a given chemical. At low exposure level for 8-h and 24-h, the Cmax-based HKAFs for benzene and styrene were greater than the HKAFs for the steady-state (*i.e.* chronic exposure) condition (*i.e.* 2 *vs* 1.6 for benzene; 3.4 *vs*. 2.5 for styrene). The opposite result was observed for 1,4-dioxane (3.1–4.0 *vs*. 6.8). Cmax-based HKAFs increased with the exposure duration at HEAEGL-1 and HEAEGL-2 for styrene as well as at HEAEGL-1 for benzene and 1,4-dioxane, but remained stable for TRI. Besides, the Cmax-based HKAFs for HEAEGL-2 decreased

significantly as compared to HEAEGL-1 for the same durations, for benzene and styrene. For the 8-h exposure condition, the Cmax-based HKAFs for benzene and styrene increased from the HEC (2 and 3.4) to HEAEGL-1 (2.4 and 5.2) and then decreased at HEAEGL-2 (1.5 and 3.4), whereas a decrease was obtained for 1,4-dioxane (3.1 to 2.5).

3.4 Discussion

This study aimed at evaluating the influence of the exposure duration and intensity on HKAFs for four VOCs with different physico/biochemical characteristics. The results suggest that the HKAFs required to protect 95 % of the individuals in the most sensitive subpopulation varies according to the duration and the intensity of the exposure to the chemicals. The present work identified the subpopulation that was the most sensitive from the TK perspective, as a function of dose metrics and scenario.

Based on the results of the present study, the adequacy of the default UF (Table 3-IV) with regard to the protection of at least 95 % of the individuals in each subpopulation may be questioned in some cases on the basis of Cmax. As per the IPCS methodology (IPCS, 2005), the interindividual UF of 10 encompasses the variability in both the TK and TD aspects (see Introduction); therefore, the results of the current study can be compared to the default TK component of 3.2 in Table 3-IV. However, such a clear-cut comparison/characterization of the TK component is not feasible when a total UF of 2 or 3 has been used in the AEGL development.

In this context, it is noteworthy that the HKAF obtained for 1,4-dioxane in neonates at steady-state condition was 6.8 (Table 3-V). Besides, the results obtained with regard to exposure to AEGL-1 for styrene raise concerns because no UF has been used in its determination (U.S. EPA, 2008). On the contrary, Table 3-V shows that a potentially high

level of variability can be attributed to AEGL-1 for the parent compound's TK alone because HKAFs of 1.2–5.2 were obtained depending of the subpopulation, duration, and dose metrics. Also, a total UF of 3 was used for the determination of benzene's AEGL-1 and styrene's AEGL-2 (U.S. EPA, 2008, 2009b). According to our results, TK variability alone would require an HKAF near or even exceeding this value in several cases. In particular, the 8-h exposure to styrene's AEGL-2 for neonates (3.4) and toddlers (2.8), as well as the 60-min and 8-h exposure to benzene's AEGL-1 for neonates (2.4 and 2.7) are noteworthy. Finally the Amet-based HKAF for TRI at HEAEGL-1 in adults and PW (2.0) as well as in toddlers for 10 min (1.5) are close or equal to the total UF used (2.0).

The HKAFs computed in this study were generally comparable to the results reported in the literature (Abraham *et al.*, 2005a; Mielke *et al.*, 2005; Mörk and Johanson, 2010; Nong *et al.*, 2006; Pelekis *et al.*, 2003; Sarangapani *et al.*, 2003; Valcke and Krishnan, 2011). This similarity can be explained by the fact that the chemicals investigated in these studies exhibit similar hepatic extraction ratios and Pb and share the same hepatic metabolism pathway (CYP2E1). Thus, HKAFs of 1.8–3.6 based on AUC for the neonates and of 1.6–2.2 based on the Amet for pregnant women were obtained in a previous work for trihalomethanes, tri- and tetra-chloroethylene (Valcke and Krishnan, 2011). In comparison, the Cmax-based HKAFs for a 24-h exposure duration in the current study varied between 1.2 and 3.4 in neonates and between 1.6 and 2.1 based on Amet in pregnant women. The results for toddlers and adults for both dose metrics were also comparable between the two studies. In addition, Nong *et al.* (2006) obtained factors of 2.5–3.9 in neonates based on the AUC for toluene and of 1.5 or 1.6 in children aged 1 mo–11 yr, whereas the current study obtained HKAFs varying between 1.1 and 2.3 based on Cmax. Greater values in the former study might be explained by the consideration of neonates as small as 0.3 kg, with corresponding sensitivity due to low development, in particular for CYP2E1 hepatic levels. For children aged 1–5 yr, a child-to-adult ratio of blood concentrations of approximately 2.3 was obtained by Pelekis *et al.* (2003) assuming

continuous exposure to dichloromethane, whereas the range of steady-state-based HKAFs in the present study for toddlers were 1.0 –2.4. Based on the distributions of the steady-state blood concentration of inhaled acetone, HKAFs of 1.5 in adults, 2.5 in 1-year toddlers, and 2.4 in 3-month-old babies were obtained by Mörk and Johanson (2010). In comparison, the HKAFs obtained in the present study were 2.1, 2.4, and 6.8 for adults, toddlers, and neonates, based on steady-state blood concentrations of 1,4-dioxane.

In the current study, the neonate-to-adult ratios of median steady-state blood concentrations were 1.4 and 1 for styrene and TRI, respectively (see Appendix). The value for styrene is slightly lower than the steady-state results reported by Sarangapani *et al.* (2003) for 1 month old babies (1.9), but the TRI value is comparable to their observation for tetrachloroethylene. For both chemicals, hepatic clearance is negligible and the steady-state blood concentration for inhalation exposure is essentially equal accross subpopulations because it is determined primarily by the Pb, which is assumed age-invariant. Thus, the lack of fitting of the TRI model with experimental data (Fig. 3.2c) would presumably not alter significantly the neonate- or toddlers- to adult ratio of internal dose metrics and corresponding HKAF. Styrene's neonate-to-adult ratio of Cmax for 8-h exposure to low exposure concentration (2.2, see Appendix) is almost the same as Abraham *et al.* (2005a)'s ratio (2.3), but is lower when considering the 8-h exposure to AEGL-2-relevant levels (2.9 *vs* 3.8). On the basis of Cmax, the neonate-to-adult ratios obtained for 8-h at low exposure level and HEAEGL-1 (1.7–2.0, see Appendix) are comparable to those obtained by Mielke *et al.* (2005) for dichloromethane (1.5–2.0). However, at HEAEGL-2 the corresponding ratios obtained for 10- and 60-min exposures were higher (3.3 and 1.5, respectively) as compared to ≈ 1.4 in Mielke *et al.* (2005).

Several determinants of toxicokinetics can contribute to explain the variations in HKAF as a function of the duration and the intensity of chemical exposure. Among these, the volume of distribution would play a pivotal role, as the neonate-to-adult ratio of Cmax for a given

acute exposure duration and intensity was function of the fat:blood partition coefficient (Pf) of the chemical (Abraham *et al.*, 2005b). In the case of styrene for example, this ratio varied from 2.2 at 1 ppm to 1.7 at 1000 ppm, peaking at 3.8 at 100 ppm; increasing the Pf two-fold diminished slightly this ratio. Comparable trend was observed with regard to Pb, but the impact of increasing it was opposite. Similarly, the three Cmax-based HKAFs obtained in the current study for 8-h exposure to increasing concentrations of styrene (Pf = 50, Pb = 52) and benzene (Pf = 54, Pb = 7.4), were 3.4, 5.0 and 4.2, and 2.0, 2.3 and 1.5, respectively. Chemical- and subpopulation-specific volume of distribution may also explain the differences in the HKAFs obtained for non steady-state exposures as compared to continuous exposures. Thus, neonate/adult differences in the proportion of body weight represented by fat may impact differently the neonate/adult ratio of arterial blood concentrations of chemicals of different liposolubility. Precisely, Fig. 3.6 shows that this ratio, in average neonates and adults, varied between 1.44 at t = 10 min and 1.36 at steady-sate (t = 12 days) with a peak at t = 60 min (1.59) for benzene. In comparison, ratios of 1.9 at t = 10 min and 3.5 at steady-state (peak, t = 10 days) were obtained for 1,4-dioxane whereas for styrene, the peak ratio was observed at t = 24 h (1440 min, 1.8). The consideration of a different dose metric for the parent compound, *i.e.* AUC, could yield different interpretation, but it is noteworthy mentioning that AUC-based HKAFs were often comparable to those obtained based on Cmax (data not shown) and that median sensitive subpopulation-to-adult ratio of both dose metrics were similar (see appendix).

Adult *vs* neonate variations in the saturation level for metabolism may also explain greater Cmax-based HKAF at HEAEGL-1 than HEAEGL-2 and HEC for benzene and styrene, and at HEC than at HEAEGL-2 for 1,4-dioxane, for an 8-h exposure. Indeed, scenarios exhibiting greater HKAF are those where the percentage of saturation is significantly greater in neonates than in adults (Fig. 3.5), yielding greater adult/neonate difference in blood concentrations. Such differences are lower when both adults and neonates either did not (HEC, Fig. 3.3) or did (HEAEGL-2, Fig. 3.4) exhibit saturation of the metabolism. At

such high levels of exposure, pulmonary clearance (which is greater in neonates due to higher alveolar ventilation rate), contributes more to overall systemic clearance, leading to the lower neonate-to-adult HKAF. Results from Abraham *et al.* (2005a, 2005b) and Mielke *et al.* (2005) also support this effect, as their neonate-to-adult ratios of blood concentrations diminished with increasing exposure concentration or increasing time of exposure at an already high exposure concentration. Besides, lower neonates' HKAF obtained for the simulation of an 8-h exposure to a concentration corresponding to 10 (the UF) × the AEGL-3 support this reasoning. Indeed, based on Cmax, values of 1.4, 2.7 and 2.4 were obtained for benzene, styrene and 1,4-dioxane, respectively (data not shown). Differences in the predominant clearance processes (Abraham *et al.*, 2005b), or combination of the effects listed above, may explain the duration- or intensity-related differences in the HKAF, but the exact mechanisms involved remain to be investigated.

In the present study, several limitations have to be acknowledged because they may constitute sources of error in the results obtained. First, the Pb was assumed to be constant across the subpopulations despite differences in tissue compositions (Clewell *et al.*, 2002; Valcke and Krishnan, 2009). However, this assumption is supported by data from Malviya and Lerman (1990). Second, the hepatic metabolism of the chemicals was considered to be mediated only by CYP2E1 enzymes, regardless of the exposure intensity. Other phase I enzymes may be involved, especially at elevated exposure levels, when the high affinity, low capacity CYP2E1 pathway is reaching saturation. In particular, other CYPs and an unidentified pathway have been associated with the metabolism of 1,4-dioxane (Nannelli *et al.*, 2005) and benzene (Rappaport *et al.*, 2009), respectively, with the latter having a lower capacity/higher affinity than CYP2E1. However, these pathways cannot be accounted for at this time given the lack of a human PBPK model to facilitate the evaluation of the interindividual variability in internal DM. Finally, the sensitive subpopulations were assumed to be healthy, and individuals with an impaired health status were not included in the present study. In particular the elderly were not considered because their sensitivity to acute exposure of VOCs is likely more related to local irritating effects to the lungs and an

overall impaired cardiovascular status, reducing their ability to escape, rather than to the systemic CNS effects underlying the AEGLs investigated.

In conclusion, this work investigated as to whether and how the HKAF varies as a function of the exposure duration and intensity, for specific sensitive subpopulations. The results of the present study have shown that the magnitude of interindividual variability in dose metrics may be within, equal to, or exceed the default UF used in establishing AEGLs. This situation can be resolved with the use of PBPK models as shown in this study to develop CSAFs for human variability in TK as a function of the duration and concentration of the human exposure guidelines.

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3.5 References

Abraham, K., Mielke, H., Huisinga W., Gundert-Remy, U. 2005a. Elevated internal exposure of children in simulated acute inhalation of volatile organic compounds: effects of concentration and duration. *Arch. Toxicol. 79*, 63-73.

Abraham, K., Mielke, H., Huisinga W., Gundert-Remy, U. 2005b. Internal exposure of children by simulated acute inhalation of volatile organic compounds: the influence of chemical properties on the child/adult concentration ratio. *Basic Clin.Pharmacol Toxicol. 96*, 242-243.

Benignus, V. A., Boyes, W. K., Kenyon, E. M., Bushnell, P. J. 1998. A dosimetric analysis of behavioural effects of acute toluene exposure in rats and humans. *Toxicol. Sci. 43*, 186-195.

Benignus, V. A., Bushnell, P. J., Boyes, W. K., Eklund, C., Kenyon, E. M. 2009. Neurobehavioral effects of acute exposure to four solvents: meta-analyses. *Toxicol. Sci. 109*, 296-305.

Boyes, W. K., Bushnell, P.J., Crofton K. M., Evans, M. 2000. Neurotoxic and pharmacokinetic response to trichloroethylene as a function of exposure scenario. *Environ Health Perspect. 108(S2)*, 317-322.

Brown, R. P., Delp, M. D., Lindstedt, S. L., Rhomberg, L. R., Beliles, R. P. 1997. Physiological parameter values for physiologically based pharmacokinetic models. *Toxicol Ind. Health. 13*, 407-484.

Clewell, H. J., Gearhart, J. M., Gentry, P. R., Covington, T. R., VanLandingham, C. B., Crump, K. S., Shipp, A. M. 1999. Evaluation of the uncertainty in an oral reference dose for methylmercury due to interindividual variability in pharmacokinetics. *Risk Anal. 19*, 547-558.

Clewell, H. J., III., Andersen, M. E., Blaauboer, B. J. 2008. On the incorporation of chemical-specific information in risk assessment. *Toxicol Lett. 180*, 100-109.

Clewell, H. J., Gentry, P. R., Covington, T. R., Sarangapani, R., Teeguarden, J. G. 2004. Evaluation of the potential impact of age- and gender-specific pharmacokinetic differences on tissue dosimetry. *Toxicol Sci. 79*, 381-393.

Clewell, H. J., Teeguarden, J., McDonald, T., Sarangapani, R., Lawrence, G., Covington, T., Gentry, R., Shipp, A. 2002. Review and evaluation of the potential impact of age- and gender-specific pharmacokinetic differences on tissue dosimetry. *Crit Rev Toxicol. 32*, 329-389.

Dorne, J. L., Renwick, A. G. 2005. The refinement of uncertainty/safety factors in risk assessment by the incorporation of data on toxicokinetic variability in humans. *Toxicol. Sci. 86*, 20-26.

Dorne, J. L., Walton, K., Renwick, A. G. 2005. Human variability in xenobiotic metabolism and pathway-related uncertainty factors for chemical risk assessment: a review. *Food Chem. Toxicol. 43*, 203-216.

Dourson, M. L., Felter, S. P., Robinson, D. 1996. Evolution of science-based uncertainty factors in noncancer risk assessment. *Regul. Toxicol. Pharmacol. 24*, 108-120.

Dourson, M. L., Stara, J. F. 1983. Regulatory history and experimental support of uncertainty (safety) factors. *Regul. Toxicol. Pharmacol. 3*, 224-238.

Faustman, E. M., Ribeiro, P. 1990. Pharmacokinetic consideration in developmental toxicity. IN: R. D. Hood (Ed.), *Developmental toxicology: risk assessment and the future*, Van Nostrand Reinhold, New York NY, pp. 109-136.

Gentry, P. R., Hack, C. E., Haber, L., Maier, A., Clewell, H. J., III. 2002. An approach for the quantitative consideration of genetic polymorphism data in chemical risk assessment: examples with warfarin and parathion. *Toxicol. Sci.* 70, 120-139.

Ginsberg, G., Hattis, D., Sonawane, B., Russ, A., Banati, P., Kozlak, M., Smolenski, S., Goble, R. 2002. Evaluation of child/adult pharmacokinetic differences from a database derived from the therapeutic drug literature. *Toxicol. Sci. 66*, 185-200.

Haddad, S., Beliveau, M., Tardif, R., Krishnan, K. 2001. A PBPK modeling-based approach to account for interactions in the health risk assessment of chemical mixtures. *Toxicol. Sci. 63*, 125-131.

Haddad, S., Pelekis, M., Krishnan, K. 1996. A methodology for solving physiologically based pharmacokinetic models without the use of simulation softwares. *Toxicol. Lett. 85*, 113-126.

Haddad, S., Restieri, C., Krishnan, K. 2001. Characterization of age-related changes in body weight and organ weights from birth to adolescence in humans. *J Toxicol. Environ Health A*. *64*, 453-464.

Haddad, S., Tardif, G. C., Tardif, R. 2006. Development of physiologically based toxicokinetic models for improving the human indoor exposure assessment to water contaminants: trichloroethylene and trihalomethanes. *J Toxicol. Environ Health A. 69*, 2095-2136.

ICRP. 2002. Annals of the ICRP. ICRP Publication 89. *Ann ICRP. 32*, 5-265.

IPCS. 2005. Chemical-Specific Adjustment Factors (CSAFs) for Interspecies Differences and Human Variability: Guidance Document for the Use of Data in Dose/Concentration-Response Assessment. World health Organization. WHO/IPCS/01.4, 1-96.

IPCS. 1994. Assessing human health risks of chemicals: Derivation of guidance values for health based exposure limits. 170. Geneva, World Health Organization, International Panel on Chemical Safety. Environmental Health Criteria. Report

Johnsrud, E. K., Koukouritaki, S. B., Divakaran, K., Brunengraber, L. L., Hines, R. N., McCarver, D. G. 2003. Human hepatic CYP2E1 expression during development. *J Pharmacol. Exp. Ther. 307*, 402-407.

Klingler, M., Demmelmair, H., Larque, E., and Koletzko, B. (2003). Analysis of FA contents in individual lipid fractions from human placental tissue. *Lipids. 38*, 561-566.

Lipscomb, J. C., Teuschler, L. K., Swartout, J., Swan, G. E., Snawder, J. E. 2003. Variance of microsomal protein and cytochrome P450 2E1 and 3A forms in adult human liver. *Toxicol. Mech. Meth. 13*, 45-51.

Lu, Y., Rieth, S., Lohitnavy, M., Dennison, J., El-Masri, H., Barton, H. A., Bruckner, J., Yang, R. S. 2008. Application of PBPK modeling in support of the derivation of toxicity reference values for 1,1,1-trichloroethane. *Regul. Toxicol Pharmacol. 50*, 249-260.

Malviya, S., Lerman, J., 1990. The blood/gas solubilities of sevoflurane, isoflurane, halothane and serum constituent concentrations in neonates and adults. *Anesthesiology 72*, 793-796.

Meek, M. E., Renwick, A., Ohanian, E., Dourson, M., Lake, B., Naumann, B. D., Vu, V. 2002. Guidelines for application of chemical-specific adjustment factors in dose/concentration-response assessment. *Toxicology. 181-182*, 115-120.

Mielke, H., Gundert, A., Abraham, K., Gundert-Remy, U. 2005. Acute inhalative exposure assessment: derivation of guideline levels with special regard to sensitive subpopulations and time scaling. *Toxicology. 214*, 256-267.

Mörk, A. K., Johanson, G. 2010. Chemical-specific adjustment factors for intraspecies variability of acetone toxicokinetics using a probabilistic approach. *Toxicol Sci. 116*, 336-348.

Nannelli, A., De, R. A., Longo, V., Gervasi, P. G. 2005. Effects of dioxane on cytochrome P450 enzymes in liver, kidney, lung and nasal mucosa of rat. *Arch. Toxicol. 79*, 74-82.

NRC. 2001. Standard operating procedures for developing Acute Exposure Guideline Levels for hazardous chemicals. National Research Council. National Academy Press. Washington DC.

Nelson, W. E. 1991. Textbook of Pediatrics, W.B. Sauders Company.

Nolan, R. J., Freshour, N. L., Rick, D. L., McCarty, L. P., Saunders, J. H. 1984. Kinetics and metabolism of inhaled methyl chloroform (1,1,1-trichloroethane) in male volunteers. *Fundam. Appl. Toxicol. 4*, 654-662.

Nong, A., Krishnan, K. 2007. Estimation of interindividual pharmacokinetic variability factor for inhaled volatile organic chemicals using a probability-bounds approach. *Regul. Toxicol. Pharmacol. 48*, 93-101.

Nong, A., McCarver, D. G., Hines, R. N., Krishnan, K. 2006. Modeling interchild differences in pharmacokinetics on the basis of subject-specific data on physiology and hepatic CYP2E1 levels: a case study with toluene. *Toxicol. Appl. Pharmacol. 214*, 78-87.

Pekari, K., Vainiotalo, S., Heikkila, P., Palotie, A., Luotamo, M., Riihimaki, V. 1992. Biological monitoring of occupational exposure to low levels of benzene. *Scand. J Work Environ Health. 18*, 317-322.

Pelekis, M., Krewski, D., Krishnan, K. 1997. Physiologically based algebraic expressions for predicting steady-state toxicokinetics of inhaled vapors. *Toxicol. Meth*. 7, 205-225.

Pelekis, M., Gephart, L. A., Lerman, S. E. 2001. Physiological-model-based derivation of the adult and child pharmacokinetic intraspecies uncertainty factors for volatile organic compounds. *Regul. Toxicol. Pharmacol. 33*, 12-20.

Pelekis, M., Nicolich, M. J., Gauthier, J. S. 2003. Probabilistic framework for the estimation of the adult and child toxicokinetic intraspecies uncertainty factors. *Risk Anal. 23*, 1239-1255.

Poulin, P., Krishnan, K. 1995. A biologically-based algorithm for predicting human tissue: blood partition coefficients of organic chemicals. *Hum. Exp. Toxicol. 14*, 273-280.

Price, P. S., Conolly, R. B., Chaisson, C. F., Gross, E. A., Young, J. S., Mathis, E. T., Tedder, D. R. 2003. Modeling interindividual variation in physiological factors used in PBPK models of humans. *Crit Rev Toxicol.* 33, 469-503.

Ramsey, J. C., Andersen, M. E. 1984. A physiologically based description of the inhalation pharmacokinetics of styrene in rats and humans. *Toxicol Appl. Pharmacol.* 73, 159-175.

Ramsey, J. C., Young, J. D., Karbowski, R. J., Chenoweth, M. B., McCarty, L. P., Braun, W. H. 1980. Pharmacokinetics of inhaled styrene in human volunteers. *Toxicol Appl. Pharmacol. 53*, 54-63.

Rappaport, S. M., Kim, S., Lan, Q., Vermeulen, R., Waidyanatha, S., Zhang, L., Li, G., Yin, S., Hayes, R. B., Rothman, N., Smith, M. T. 2009. Evidence that humans metabolize benzene via two pathways. *Environ Health Perspect. 117*, 946-952.

Reitz, R. H., McCroskey, P. S., Park, C. N., Andersen, M. E., Gargas, M. L. 1990. Development of a physiologically based pharmacokinetic model for risk assessment with 1,4-dioxane. *Toxicol Appl. Pharmacol. 105*, 37-54.

Renwick, A. G., Lazarus, N. R. 1998. Human variability and noncancer risk assessment--an analysis of the default uncertainty factor. *Regul. Toxicol. Pharmacol. 27*, 3-20.

Ronis, M. J. J., Lindros, K. O., Ingelman-Sundberg, M. 1996. The CYP2E family. IN: C. Ioannides (Ed.), *Cytochrome P450 Metabolic and Toxicological Aspects*, CRC Press, Boca Raton FL, pp. 211-240.

Sarangapani, R., Gentry, P. R., Covington, T. R., Teeguarden, J. G., Clewell, H. J., III. 2003. Evaluation of the potential impact of age- and gender-specific lung morphology and ventilation rate on the dosimetry of vapors. *Inhal. Toxicol. 15*, 987-1016.

Statistics Canada. 2003. Canadian community health survey, cycle 2.1. Statistics Canada.

Sweeney, L. M., Thrall, K. D., Poet, T. S., Corley, R. A., Weber, T. J., Locey, B. J., Clarkson, J., Sager, S., Gargas, M. L. 2008. Physiologically based pharmacokinetic modeling of 1,4-Dioxane in rats, mice, and humans. *Toxicol. Sci. 101*, 32-50.

Tan, Y. M., Liao, K. H., Clewell, H. J., III. 2007. Reverse dosimetry: interpreting trihalomethanes biomonitoring data using physiologically based pharmacokinetic modeling. *J Expo. Sci. Environ Epidemiol. 17*, 591-603.

Taniguchi, H., Oguro, A., Koyama, H., Masuyama, M., and Takahashi, T. 1996. Analysis of models for quantification of arterial and portal blood flow in the human liver using PET. *J Comput. Assist. Tomogr. 20*, 135-144.

Thomas, R. S., Bigelow, P. L., Keefe, T. J., Yang, R. S. 1996. Variability in biological exposure indices using physiologically based pharmacokinetic modeling and Monte Carlo simulation. *Am Ind. Hyg. Assoc. J. 57*, 23-32.

U.S. EPA. 2000. Interim Acute Exposure Guidelines (AEGLs) - 1,1,1-Trichloroethane. U.S. Environmental Protection Agency. Washington DC.

U.S. EPA. 2002. A review of the reference dose and reference concentration process. Risk Assessment Forum. EPA/630/P-02/00F. Washington DC, U.S. Environmental Protection Agency.

U.S. EPA. 2005. Interim Acute Exposure Guidelines (AEGLs) - 1,4-dioxane. U.S. Environmental Protection Agency. Washington DC.

U.S. EPA 2008. Interim Acute Exposure Guidelines (AEGLs) - Styrene. U.S. Environmental Protection Agency. Washington DC.

U.S. EPA. 2009a. Integrated Risk Information System. U.S. Environmental Protection Agency, Washington DC.

U.S. EPA. 2009b. Interim Acute Exposure Guidelines (AEGLs) - Benzene. U.S. Environmental Protection Agency. Washington DC

Valcke, M., Krishnan, K. 2009. Physiologically Based Pharmacokinetic Modeling in the Risk assessment of Developmental Toxicants. IN: D.K.Hansen, B.D. Abbott (Eds.), *Developmental Toxicology*, Informa Health Care, New York NY, pp. 243-274.

Valcke, M., Krishnan, K. 2011. Evaluation of the Impact of the Exposure Route on the Human Kinetic Adjustment Factor. *Regul. Toxicol Pharmacol, 59,* 258-269.

Wang, Y., Moss, J., Thisted, R. 1992. Predictors of body surface area. *J Clin Anesth. 4*, 4-10.

WHO. 2005. 1,4-Dioxane in drinking-water. Background document for development of WHO Guidelines for drinking-water quality. Geneva, World Health Organization (WHO/SDE/WSH/05.08/120).

FIGURE CAPTIONS

Figure 3.1 : Conceptual representation of the inhalation PBPK models for benzene, styrene, 1,1,1-trichloroethane, and 1,4-dioxane. Dotted lines show the additional compartments that represent pregnant women.

Figure 3.2 : Evaluation of the PBPK models by comparison of the simulated kinetics with the experimental data following inhalation exposure to benzene (a), reported by Pekari *et al.* (1992); styrene (b), reported by Ramsey *et al.* (1980); 1,1,1-trichloroethane (c), reported by Nolan *et al.* (1984); and 1,4-dioxane (d), reported by Sweeney *et al.* (2008).

Figure 3.3 : 24-h model simulation of the arterial blood concentration in average adult (ad), neonate (neo), toddler (todd) and pregnant woman (PW), for an 8-h inhalation exposure to an HEC of benzene (a), styrene (b), 1,1,1-trichloroethane (c), and 1,4-dioxane (d).

Figure 3.4 : 24-h model simulation of the arterial blood concentration and rate of metabolism in average adult (ad) and neonate (neo), for an 8-h inhalation exposure to HEAEGL-2 for benzene (a) styrene (b) 1,1,1-trichloroethane (c) and 1,4-dioxane (d). Saturation is observed for benzene, styrene and 1,4-dioxane, but did not occur for 1,1,1-trichloroethane at any exposure scenario.

Figure 3.5 : 24-h model simulation of the arterial blood concentration and rate of metabolism in average adult (ad) and neonate (neo), for an 8-h inhalation exposure to HEAEGL-1 for benzene (a) styrene (b).

Figure 3.6 : Simulation of arterial blood concentration in average adult and neonate during continuous exposure to HEC of benzene (a), styrene (b) and 1,4-dioxane (c) until steady-state is reached.

Figure 3.1

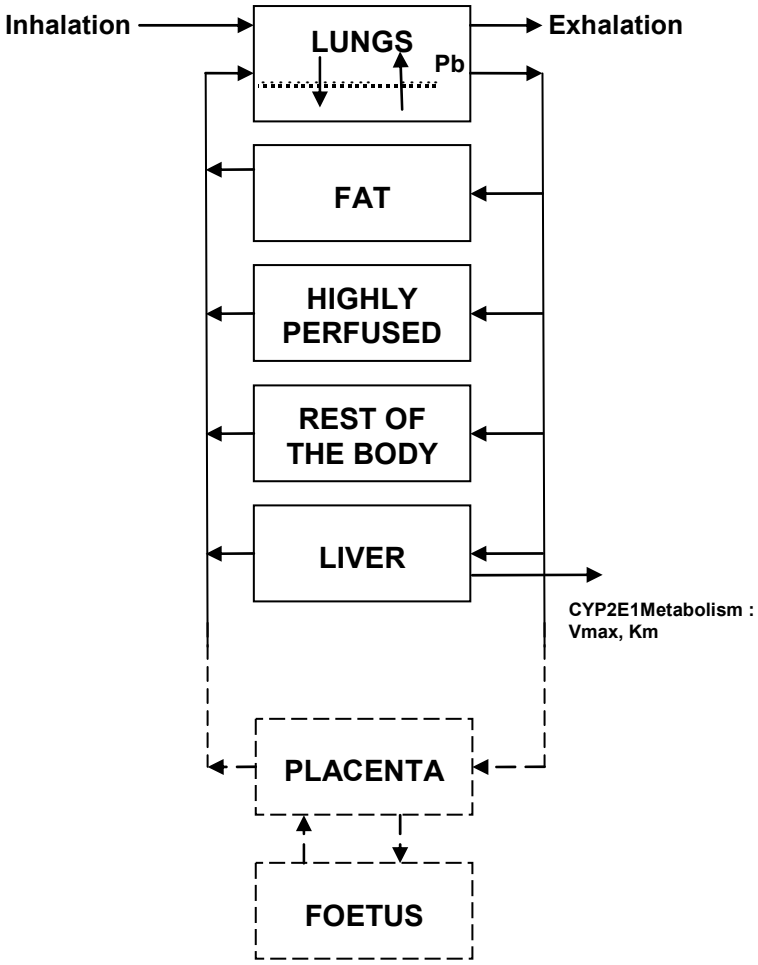
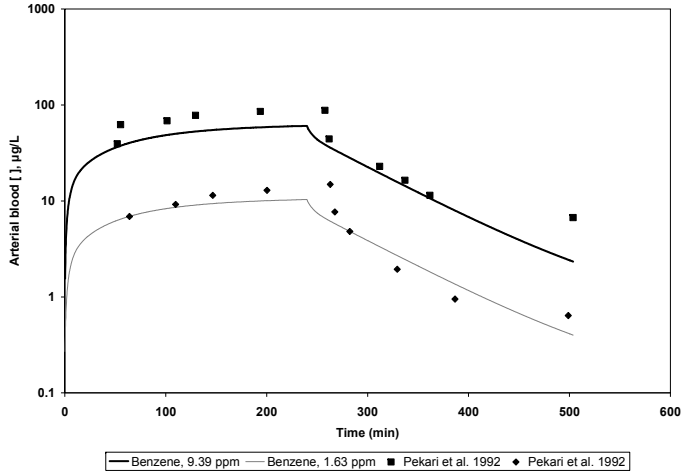

Inhalation
LUNGS
Pb
Exhalation
FAT
HIGHLY PERFUSED
REST OF THE BODY
LIVER
CYP2E1Metabolism : Vmax, Km
PLACENTA
FOETUS

Figure 3.2

a)



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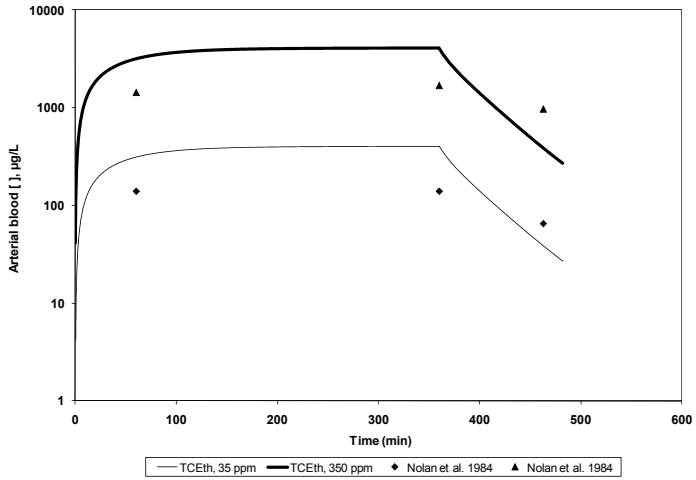

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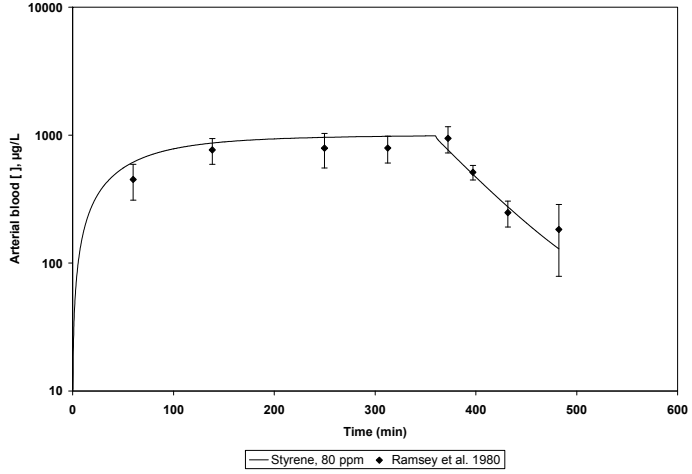

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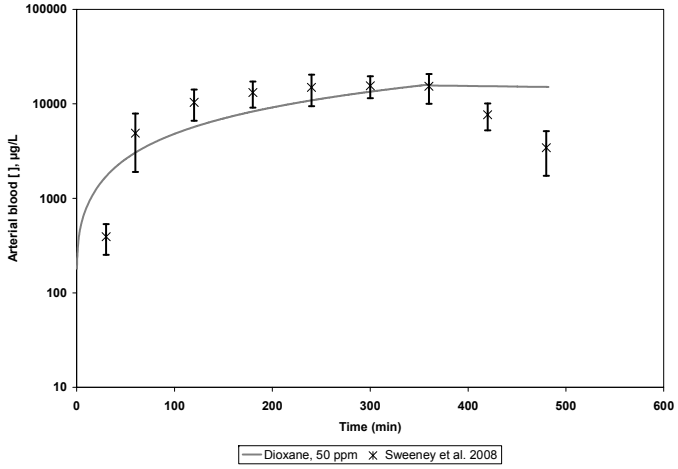
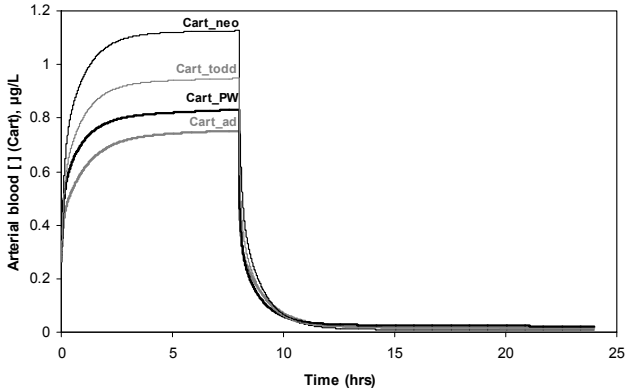


Figure 3.3

a)



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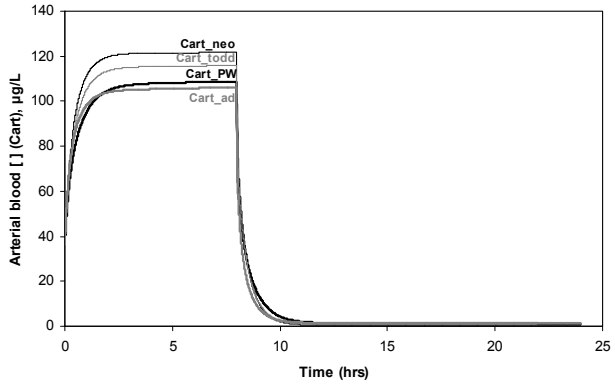

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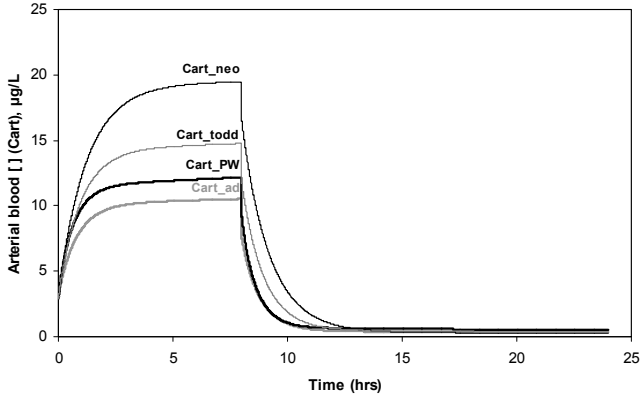


d)

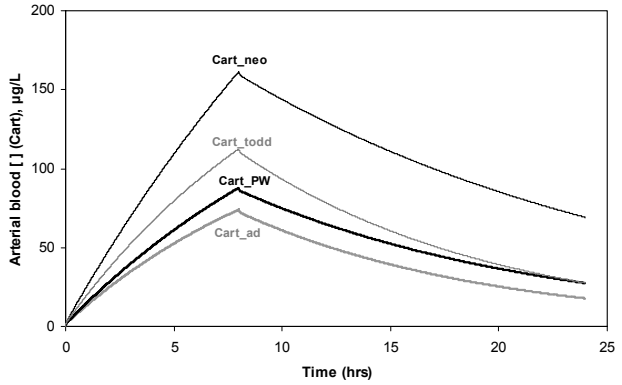
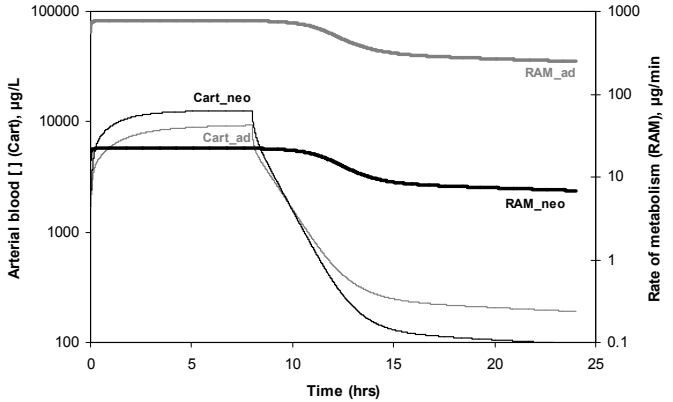
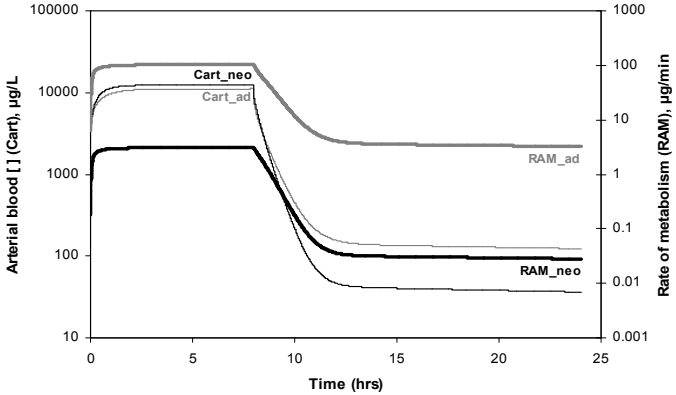


Figure 3.4

a)



c)



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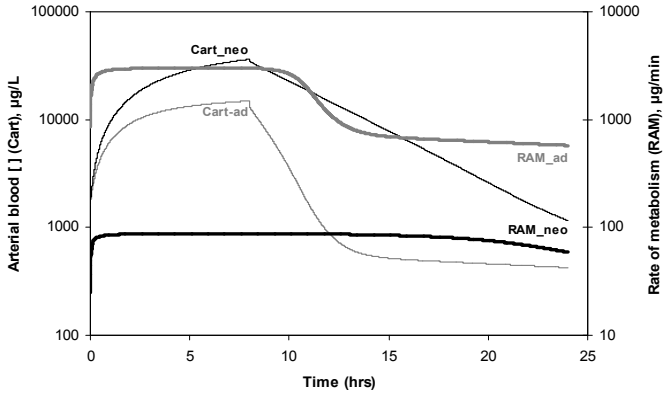


d)

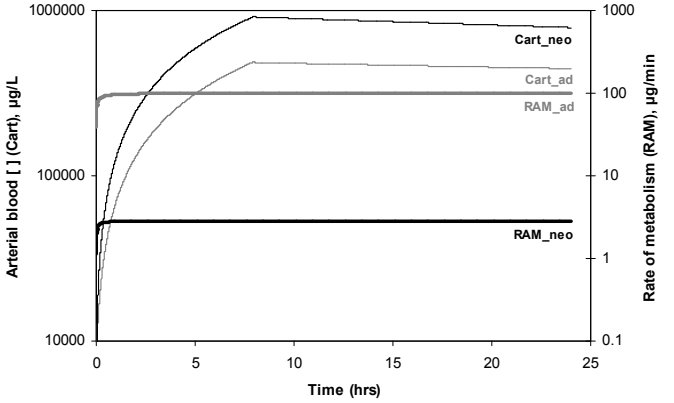
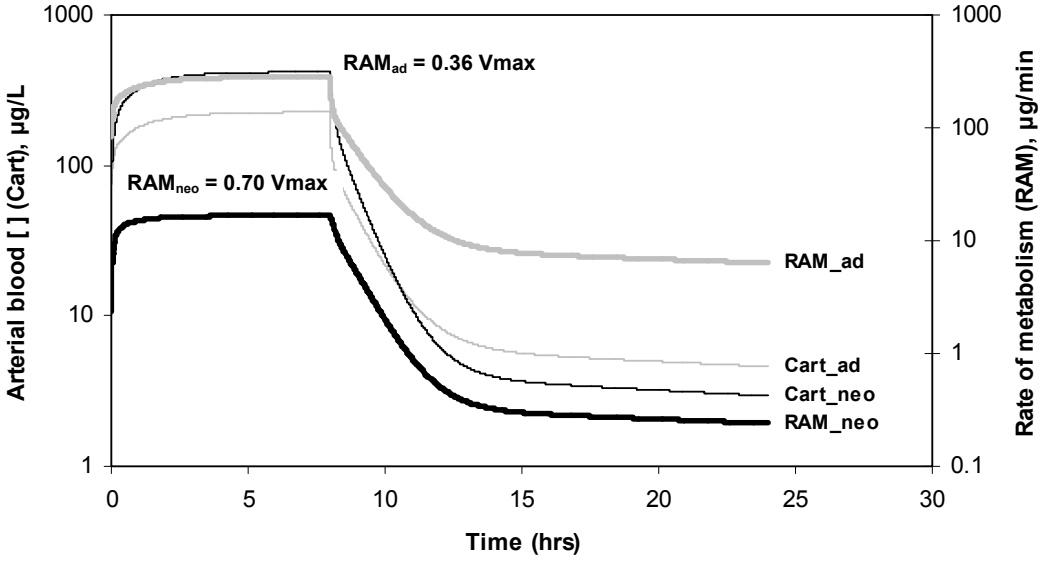


Figure 3.5

a)


1000
100
10
1
0.1
0
5
10
15
20
25
30
Time (hrs)
Arterial blood [ ] (Cart), µg/L
Rate of metabolism (RAM), µg/min
RAM_ad = 0.36 Vmax
RAM_neo = 0.70 Vmax
RAM_ad
Cart_ad
Cart_neo
RAM_neo

b)

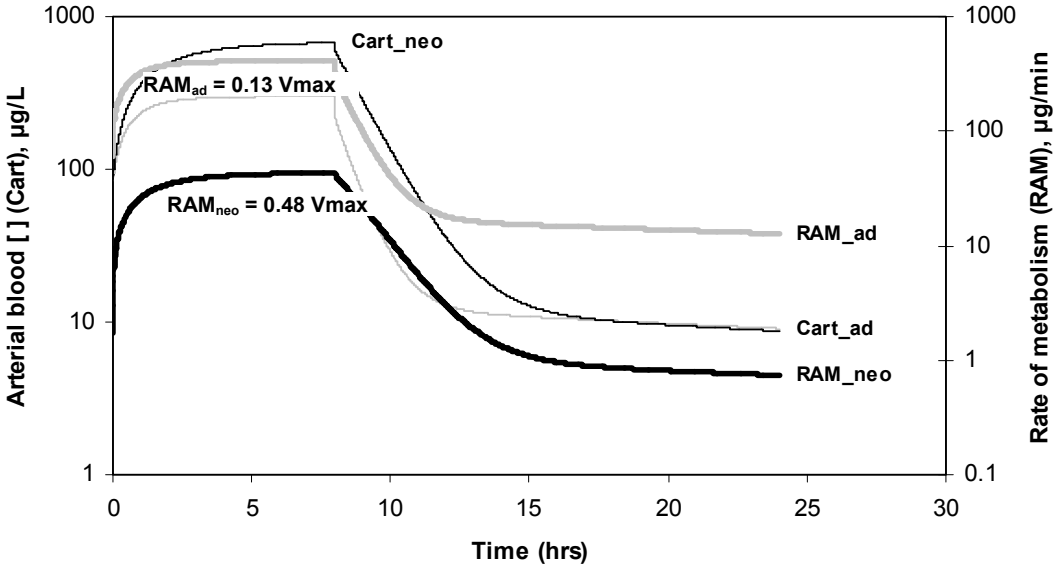
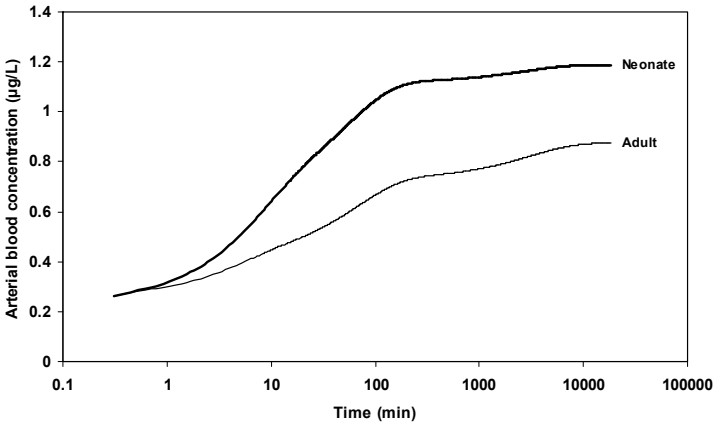
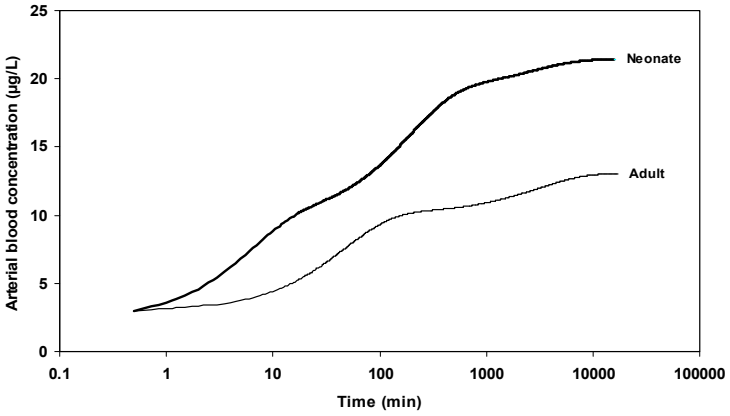

1000
100
10
1
0.1
0
5
10
15
20
25
30
Time (hrs)
Arterial blood [ ] (Cart), µg/L
Rate of metabolism (RAM), µg/min
Cart_neo
RAM_ad = 0.13 Vmax
RAM_neo = 0.48 Vmax
RAM_ad
Cart_ad
RAM_neo

Figure 3.6

a)



b)



c)

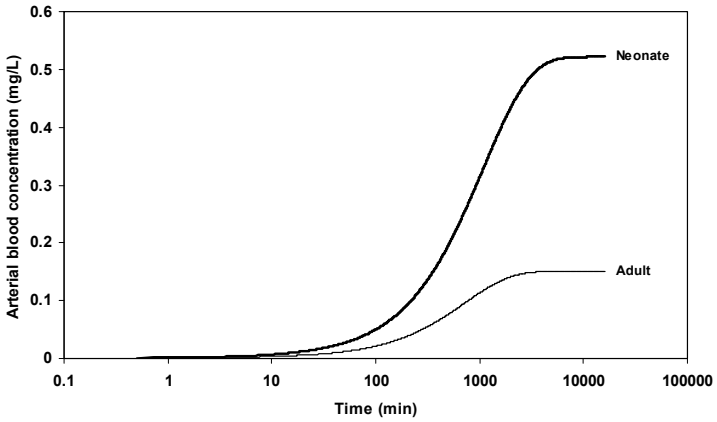


Table 3-I : Chemical-specific parameters for PBPK modeling

Parameters	Chemical			
	Benzene[a)]	Styrene[b)]	1,1,1-Trichloroethane[c)]	1,4-dioxane[d)]
Molecular weight (g/mol)	78.1	104.2	133.4	88.1
Partition coefficients				
Blood:air	7.4	52	2.53	3650
Liver:air[e)]	11	140.5	8.6	1570
Fat:air[e)]	396	2600	263	840
Highly perfused tissues:air[e)]	11	296.4	8.6	1570
Rest of the body:air[e)]	15	52	3.16	1570
Placenta:blood[f)]	1.28	2.16	1.69	1.1
Metabolic constants				
Maximal rate (μg/min-kg$^{0.75}$)	35.2	139.3	7	4.5
Michaelis-Menten (μg/L)	100	360	5750	3000

Parameters values were taken from a) Haddad *et al.* (2001a); b) Ramsey and Andersen (1984); c) Lu *et al.* (2008); and d) Reitz *et al.* (1990). e) The tissue:blood partition coefficients are calculated in the models as the tissue:air partition coefficients divided by the blood:air partition coefficient. f) Calculated according to Poulin and Krishnan (1995), based on the composition of the placenta as described by Klingler *et al.* (2003).

Table 3-II : Equations used to calculate the physiological parameters in the PBPK models for adults (ad), neonates (neo), toddlers (todd), and pregnant women (PW) using the data on body weight (BW), body height (BH) and age.

Parameter Subpopulation	Equation	Reference
Body surface area (SA, cm^2)		
ad, neo, todd	$= BW^{0.515} \times BH^{0.422} \times 234.9$	Haddad *et al.*, 2006
PW	$= BW^{0.425} \times BH^{0.725} \times 71.84$	Wang *et al.*, 1992
Tissue volumes (L)		
Liver (Vl)		
ad, PW	$= 0.026 \times BW$	Brown *et al.*, 1997
neo, todd	$= 0.05012 \times BW^{0.78}$	Haddad *et al.*, 2006
Highly perfused (Vh)[a)]		Haddad *et al.*, 2006
ad, PW	$= [(-5.309E\text{-}3 \times age + 1.008 \times BW^{0.493} + BH^{0.3765} - 7.952) + (-2.331E\text{-}3 \times age + 0.1253 \times BW^{0.8477} + BH^{0.3821} - 4.725)]/2$	
neo, todd	$= [(-1.0682E\text{-}2 \times age + 2.038 \times (BW^2 / BH)^{0.4014} - 0.2046) + (-1.919E\text{-}2 \times age + 3.193 \times (BW^2 / BH)^{0.2657} - 1.374)]/2$	
Fat (Vf)		
ad	$= [((1.36 \times BW / (BH / 100)) - 42) + ((1.61 \times BW / (BH / 100)) - 38.3)]/2$	Price *et al.*, 2003
todd	$= [((908.4 + 0.706 \times (BW \times 1000) - 53 \times BH - 3.057 \times (age \times 365.25)) / 1000) + ((908.4 + 0.706 \times (BW \times 1000) - 53 \times BH + 358.5 - 3.057 \times (age \times 365.25)) / 1000)]/2$	Price *et al.*, 2003
neo[b)]	$= 0.14 \times BW$	Haddad *et al.*, 2001b
PW[c)]	$= [1.61 \times BW / (BH / 100)] - 38.3 + 3.825$	Price *et al.*, 2003; ICRP, 2002
Bones and skeleton (Vb)		
ad, PW[d)]	$= 0.09 \times BW$	Tan *et al.*, 2007

neo, todd[e]	= [[[(-0.0306 × age^5 + 0.5222 × age^4 + 9.7109 × age^3-187.97 × age^2 + 1089.7 × age + 546.6) / 1000] - [(0.0019956 × age^6 - 0.11169 × age^5 + 2.189 × age^4 -17.726 × age^3 + 59.767 × age^2 + 14.405 × age + 73.716) / 1000]] + [[(-0.002831 ×age^5 + 0.18184 × age^4 + 10.685 × age^3-142.88 × age^2 + 782.05 × age + 609.64) / 1000] - [(0.0007984 × age^6 - 0.037966 × age^5 + 0.5272 × age^4 - 1.1311 × age^3 -12.285 × age^2 + 123.87 × age + 53.358) / 1000]]]/2	Haddad *et al.*, 2001b
Foeto-placental unit (Vpl)		
fetus only	= 0.0618 × BW_{PW}	ICRP, 2002
	= 0.677 × Vpl	ICRP, 2002
Rest of the body (Vr)		
ad, neo, todd		
PW	= BW - (Vb + Vf + Vh + Vl) = BW - (Vpl + Vb + Vf + Vh + Vl)	Haddad *et al.*, 2006
Blood Flows (L/min)		
Cardiac output (Qc)		
Ad, neo, todd	= [(0.2519 × BW$^{0.7609}$) + (0.2508 × BW$^{0.7815}$)] / 2	Haddad *et al.*, 2006
PW[f]	= (0.2508 × BW$^{0.7815}$) × 1.346	Haddad *et al.*, 2006; ICRP, 2002
Liver (Ql)	= 0.92 × Vl	Haddad *et al.*, 2006
Fat (Qf)	= 0.025 × Vf	Haddad *et al.*, 2006
Foeto-placental unit (Qpl)		
	= 0.12 × Qc	ICRP, 2002
fetus only	= 0.017	Clewell *et al.*, 1999
Rest of the body (Qr)	g)	Haddad *et al.*, 2006
Highly perfused (Qh)		
ad, neo, todd	= Qc - (Qf + Qr + Ql)	
PW	= Qc - (Qpl + Qf + Qr + Ql)	
Alveolar ventilation (Qalv, L/min)	= Qc	Haddad *et al.*, 2006

a) The volumes of liver were subtracted from the result of this equation to obtain the volume of the highly perfused tissue in the PBPK models. b) The data presented by Haddad *et al.* (2001b) show that the adipose tissue represents roughly 14 % of the neonates' body weight when born at term. c) Equation of Price *et al.* (2003), for non-pregnant women, to which the mean fat weight gain during pregnancy, as reported by ICRP (2002), was added. d) For the pregnant women, the volume of the bone was based on the BW of a non-pregnant woman. e) The volume of the bones was computed as the difference between the total volume of bone in addition to the marrow and the volume of marrow alone, both of which were calculated as per Haddad *et al.* (2001b). f) The cardiac output of the non-pregnant women was increased by a factor of 1.346 based on the mean increase of the cardiac output at the 38^{th} week of pregnancy, as per ICRP (2002). g) The blood flows to the rest of the body were calculated based on the blood flows to the tongue, the heart, the skin, and the skeletal muscles and were increased by a factor that corresponds to the ratio of the volume of the rest of the body (Vr) on the sum of the volumes of these tissues. Calculations of the volumes and blood flows for these tissues were conducted as per Haddad *et al.* (2006, 2001b).

Table 3-III : Probability density functions of the descriptors that were used to define the subpopulation-specific parameters in the PBPK models

Subpopulation Median age (range) **Parameter**[b]	**Adults**[a] 41 (18–64)[a]	**Neonates**[a] 14 d (0–30 d)[a]	**Toddlers**[a] 2 (1–3)[a]	**Pregnant women**[a] 29 (15–44)[a]
Body weight (kg, mean ± SD, range):	76 ± 17, 37–152	4 ± 1, 2–7	13 ± 2, 7–32	82 ± 18, 48–166
Body height (cm, mean ± SD, range):	167 ± 10, 144–198	46 ± 16, 35–80	87 ± 6, 70–106	161 ± 7, 132–182
CYP2E1 concentration (pmol/mg MSP, mean ± SD):	49 ± 2, 11–130[c]	18 ± 14, 1–56	42 ± 18, 18–74	d)

a) See Valcke and Krishnan (2011) for details. b) Log-normally distributed. c) Geometric mean ± geometric standard deviation. d) Same as for adults. **Abbreviations**: CYP2E1, cytochrome p-450 2E1; MSP, microsomal protein; SD, standard deviation.

Table 3-IV : Levels and durations of exposure simulated by PBPK modeling

Level and duration	Chemical			
	Benzene[a)]	Styrene[b)]	1,1,1-Trichloroethane[c)]	1,4-dioxane[d)]
Low exposure[e)]				
UF for RfC	10	3	10	10
RfC, (mg/m^3)	0.030	1	5	0.075[f)]
Simulated HEC, (mg/m^3)[g)]	**0.3**	**3**	**50**	**0.75**
High exposure: AEGL1				
UF for AEGL1	3	1	2	i)
AEGL1 (ppm)[h)]	9, 52, 130	20	230	i)
Simulated HEAEGL-1 (ppm)[j)]	**27, 156, 390**	**20**	**460**	i)
High exposure: AEGL2				
UF for AEGL2	3	3	3	10
AEGL2 10 min (ppm)	2000	230	930	580
AEGL2 60 min (ppm)	800	130	600	320
AEGL2 8 h (ppm)	200	130	310	100
Simulated HEAEGL-2 10 min (ppm)[j)]	**6000**	**690**	**2790**	**5800**
Simulated HEAEGL-2 60 min (ppm)[j)]	**2400**	**390**	**1800**	**3200**
Simulated HEAEGL-2 8 h (ppm)[j)]	**600**	**390**	**930**	**1000**

a) U.S. EPA (2009b). b) U.S. EPA (2008). c) U.S. EPA (2000). d) U.S. EPA (2005). e) All reference concentrations values ("RfC") were taken from the U.S. EPA (2009a) IRIS website, except f), which was derived from the World health organisation's tolerable daily intake of 16 µg/kg-d, as described in WHO (2005), assuming a 70 kg adult inhaling 15 m^3/d. g) HEC: human equivalent concentration, which was obtained by multiplying the RfC and the uncertainty factor. h) Different AEGL1 values for 10 min, 60 min and 8 h were available for benzene only; AEGL-1 values for other substances were equal, regardless of the duration considered. i) Not simulated given that this AEGL is based on local irritating effects. j) HEAEGL: Human equivalent acute exposure guideline level, which was obtained by multiplying the AEGL and the UF.

Abbreviations: AEGL, acute exposure guideline. HEAEGL, human equivalent acute exposure guideline. HEC, Human equivalent concentration. RfC, reference concentration. UF, interindividual uncertainty factor.

Table 3-V : Subpopulation-specific HKAFs based on distributions of each dose metrics, for various conditions of exposure[a)]

Exposure intensity	Low exposure (HEC)			High exposure					
				HEAEGL-1[b)]			HEAEGL-2		
Duration / Chemical / Dose metrics	8 h	24 h	Steady-state[c)]	10 min	60 min	8 h	10 min	60 min	8 h
Benzene									
Cmax									
Adults	1.3	1.3	1.2	1.2	1.6	1.3	1.2	1.1	1.3
Neonates	**2.0**	**2.0**	**1.6**	**1.7**	**2.7**	**2.4**	**1.6**	**1.7**	**1.5**
Toddlers	1.6	1.5	1.2	1.4	2.2	1.7	1.3	1.5	1.4
Pregnant Women	1.5	1.5	1.4	1.4	1.9	1.5	1.3	1.3	1.5
Amet									
Adults	1.3	1.3	1.2	1.4	1.4	1.3	1.4	1.5	1.7
Neonates	1.4	1.4	1.1	1.1	1.0	1.2	0.7	0.6	0.7
Toddlers	1.5	1.5	1.2	1.4	1.3	1.5	1.1	1.1	1.1
Pregnant Women	**1.6**	**1.6**	**1.5**	**1.6**	**1.5**	**1.6**	**1.5**	**1.5**	**1.7**
Styrene									
Cmax									
Adults	1.5	1.5	1.4	1.2	1.3	1.5	1.2	1.3	2.0
Neonates	**3.4**	**3.4**	**2.5**	**1.5**	**2.4**	**5.2**	**1.4**	**2.1**	**3.4**
Toddlers	2.0	1.9	1.5	1.3	1.6	2.1	1.3	1.7	2.8
Pregnant Women	1.7	1.8	1.8	1.4	1.5	1.8	1.4	1.6	2.0
Amet									
Adults	1.4	1.4	1.4	1.4	1.4	1.4	1.1	1.4	1.5
Neonates	**1.8**	**1.8**	1.3	1.8	1.8	1.7	1.2	1.5	1.1
Toddlers	1.8	1.8	1.4	**1.8**	**2.0**	1.8	**1.3**	1.7	1.4
Pregnant Women	1.8	1.8	**1.9**	1.8	1.8	**1.8**	1.3	**1.7**	**1.7**

1,1,1-Trichloroethane									
Cmax									
Adults	1.1	1.1	1.0	1.1	1.1	1.1	1.1	1.1	1.1
Neonates	**1.2**	**1.2**	**1.0**	**1.3**	**1.2**	**1.1**	**1.3**	**1.2**	**1.2**
Toddlers	1.1	1.1	1.0	1.2	1.2	1.1	1.2	1.2	1.2
Pregnant Women	1.1	1.1	1.0	1.2	1.1	1.1	1.2	1.1	1.1
Amet									
Adults	2.0	2.0	**2.0**	**2.0**	2.0	2.0	**2.1**	**2.1**	**2.0**
Neonates	0.3	0.9	0.8	0.9	0.9	0.8	0.9	0.7	0.8
Toddlers	1.4	1.5	1.4	1.5	1.4	1.4	1.4	1.3	1.3
Pregnant Women	**2.1**	**2.1**	2.0	2.0	**2.0**	**2.0**	1.9	1.9	1.9
1,4-Dioxane									
Cmax									
Adults	1.3	1.6	2.1	-	-	-	1.2	1.2	1.2
Neonates	**3.1**	**4.0**	**6.8**	-	-	-	**2.2**	**2.5**	**2.5**
Toddlers	2.0	2.3	2.4	-	-	-	1.6	1.8	1.8
Pregnant Women	1.5	1.8	2.8	-	-	-	1.3	1.3	1.3
Amet									
Adults	1.4	1.4	1.4	-	-	-	2.0	**2.1**	**2.1**
Neonates	1.5	1.5	1.2	-	-	-	0.4	0.8	0.9
Toddlers	1.7	1.8	1.4	-	-	-	1.3	1.4	1.4
Pregnant Women	**1.8**	**1.9**	**1.9**	-	-	-	**2.0**	2.0	2.0

a) The actual highest HKAF obtained, based on two significant figures, is indicated in bold.

b) HEAEGL-1 for 1,4-dioxane was not simulated because it is based on local irritating effects.

c) For steady-state conditions, the dose metrics are blood concentration (μg/L) and rate of metabolism (μg/h-L of liver) instead of AUC and Amet.

Abbreviations: Amet, amount metabolized; AUC, Area under the curve; Cmax, maximum blood concentration; HEAEGL, Human equivalent acute exposure guideline level; HEC, Human equivalent concentration.

Appendix : Median internal dose metrics in each subpopulation and for each exposure scenario

Exposure conditions / Chemical / Dose metric	Low exposure			High exposure					
Level	HEC			HEAEGL-1			HEAEGL-2		
Duration	8 h	24 h	steady-state[a)]	10 min	60 min	8 h	10 min	60 min	8 h
Benzene									
AUC (min. µg/L); Cmax (µg/L)									
Adults	0.4; 0.7	1.1; 0.8	1.1	41; 2.3[b)]	98; 965	112; 226	861; 39[b)]	2.2[b)]; 25[b)]	4.4[b)]; 8.9[b)]
Neonates	0.6; 1.2	1.7; 1.2	1.3	69; 3.2[b)]	175; 2.3[b)]	214; 451	63[b)]; 129[b)]	3.1[b)]; 38[b)]	6.1[b)]; 13[b)]
Toddlers	0.5; 1.0	1.4; 1.0	1.2	54; 2.8[b)]	135; 1.8[b)]	149; 307	1.1[b)]; 45[b)]	2.7[b;] 32[b)]	5.4[b)] 11 [b)]
Pregnant women	0.4; 0.9	1.2; 0.9	1.1	47; 2.7[b)]	113; 1.5[b)]	128; 257	916; 43[b)]	2.3[b)]; 27[b)]	4.5[b)]; 9.1[b)]
Amet (µg/L of liver)									
Adults	0.3	0.8	46	18	45	74	131	225	405
Neonates	0.3	0.9	37	12	22	49	48	70	126
Toddlers	0.3	1.0	45	20	43	86	105	172	313
Pregnant women	0.3	1.0	56	20	48	89	132	235	428
Styrene									
AUC (min. µg/L); Cmax (µg/L)									
Adults	5; 10	15; 11	17	3; 128	19; 232	155; 305	128; 5.0[b)]	519; 6.2[b)]	6.1[b)]; 13[b)]
Neonates	11; 22	30; 22	25	6; 154	41; 390	385; 814	372; 5.9[b)]	1.8[b)]; 9.8[b)]	21[b)]; 37[b)]
Toddlers	8; 15	21; 16	22	5; 138	28; 305	221; 447	197; 5.4[b)]	908; 8.0[b)]	12[b);] 24[b)]
Pregnant women	6; 13	18; 14	18	4; 144	23; 281	188; 367	164; 5.6[b)]	670; 7.6[b)]	7.3[b)]; 15[b)]
Amet (µg/L of liver)									
Adults	3.9	11	770	2.3	14	111	108	239	1.3[b)]
Neonates	5.4	15	740	3.3	19	141	91	232	681
Toddlers	5.6	15	777	3.3	20	153	105	305	1.3[b)]
Pregnant women	5.2	15	1.0[b)]	3.1	18	145	97	285	1.5[b)]

1,1,1-Trichloroethane									
AUC (min. μg/L); Cmax (μg/L)									
Adults	52; 107	152; 109	126	55; 3.0[b)]	329; 4.7[b)]	2.7[b)]; 5.4[b)]	335; 18[b)]	1.3[b)]; 19[b)]	5.3[b)]; 11[b)]
Neonates	59; 122	173; 122	126	62; 3.3[b)]	369; 5.6[b)]	3.0[b)]; 6.9[b)]	373; 20[b)]	1.5[b)]; 22[b)]	6.0[b)]; 12[b)]
Toddlers	56; 116	166; 118	126	59; 3.2[b)]	354; 5.3[b)]	2.8[b)]; 5.8[b)]	359; 19[b)]	1.4[b)]; 21[b)]	5.7[b)]; 12[b)]
Pregnant women	52; 107	153; 110	126	55; 3.3[b)]	330; 4.9[b)]	2.6[b)]; 5.4[b)]	334; 20[b)]	1.3[b)]; 19[b)]	5.3[b)]; 11[b)]
Amet (μg/L of liver)									
Adults	0.8	2.1	103	0.7	3.3	22	2.6	7.4	31
Neonates	0.7	1.1	29	0.2	1.0	6	0.8	2.1	8.9
Toddlers	0.6	1.9	80	0.6	2.6	17	2.1	5.6	24
Pregnant women	0.8	2.2	103	0.7	3.2	21	2.6	6.9	29
1,4-Dioxane									
AUC (min. μg/L); Cmax (μg/L)									
Adults	57; 73	124; 131	199	331; 927	2.5[b)]; 3.4[b)]	265[b)]; 24[b)]	78[b)]; 107[b)]	262[b)]; 224[b)]	570[b)]; 494[b)]
Neonates	155; 164	311; 372	628	959; 1.5[b)]	6.6[b)]; 6.5[b)]	52[b)]; 47[b)]	147[b)] 174[b)]	492[b)]; 417[b)]	1.1[c)]; 933[b)]
Toddlers	93; 115	197; 214	277	550; 1.2[b)]	4.3[b)]; 5.1[b)]	36[b)]; 39[b)]	116[b)]; 142[b)]	385[b)]; 326[b)]	839[b)]; 737[b)]
Pregnant women	70; 85	148; 162	259	420; 958	3.1[b)]; 3.8[b)]	29[b)]; 26[b)]	86[b)]; 112[b)]	286[b)]; 245[b)]	618[b)]; 537[b)]
Amet (μg/L of liver)									
Adults	1.0	2.1	201	5.3	27	64	74	77	77
Neonates	0.8	1.5	168	4.0	13	20	22	22	22
Toddlers	1.2	2.6	200	6.5	30	54	59	63	63
Pregnant women	1.3	2.6	273	6.6	32	65	75	77	77

a) For steady-state, dose metrics are blood concentration (μg/L) and rate of metabolism (μg/h-L of liver), instead of Cmax and Amet. No Cmax is obtained. b) Mass unit in mg. c) In min-g/L.

Abbreviations: Amet, amount metabolized. Cmax, maximum blood concentration. HEAEGL, human equivalent acute exposure guideline level. HEC, human equivalent concentration.

4 Article III: *An assessment of the impact of physico-chemical and biochemical characteristics on the human kinetic adjustment factor for systemic toxicants*

Valcke, M. et Krishnan, K.

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AN ASSESSMENT OF THE IMPACT OF PHYSICO-CHEMICAL AND BIOCHEMICAL CHARACTERISTICS ON THE HUMAN KINETIC ADJUSTMENT FACTOR FOR SYSTEMIC TOXICANTS

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Abstract

The objective of this study was to evaluate the magnitude of the human kinetic adjustment factor (HKAF) as a function of physico- and bio-chemical characteristics impacting the systemic clearance of chemicals. This factor is intended to replace the default value of 3.16 in non-cancer risk assessments and aims at accounting for interindividual variability in toxicokinetics. A steady-state algorithm was used to compute the internal dose metrics (blood concentration (C_{blood}) and rate of metabolite produced/L liver (RAM)) of hypothetical chemicals in neonates, adults, elderly, and pregnant women. After evaluating the algorithm with chemical-specific experimental data, C_{blood} and RAM were calculated for hypothetical chemicals exhibiting blood:air partition coefficients (Pb) between 1 and 10,000 and hepatic extraction ratios in the average adult (E) between 0.01 and 0.99. Based on Monte Carlo simulation results, HKAF values were computed as the ratio of the 95^{th} percentile value for each subpopulation to the 50^{th} percentile value in adults. The highest HKAF among those obtained for each subpopulation was reported in route-, pathway-, and dose metric-specific HKAF matrices as a function of Pb and E. These matrices allowed the recognition of cases where the default HKAF could be exceeded, and these occurred in neonates based on C_{blood} in two situations. First, when the average adult-to-neonate ratio of body weight-adjusted systemic clearance was at least equal to 2.2 for a given systemic exposure (*i.e.*, for CYP1A2 substrates only). Second, when E = 0.01–0.2 and Pb $\geq$ 300 or when E = 0.3–0.7 and Pb $\geq$ 100 for inhalation exposures to CYP2E1 substrates, with comparable values for the other substrates (higher for CYP1A2). Overall, this study showed the dependency of the HKAF on the dose metrics, chemical characteristics, metabolic pathways, and subpopulations considered.

Keywords: human kinetic adjustment factor (HKAF), interindividual variability, Monte Carlo simulations, phase I metabolism, risk assessment, steady-state toxicokinetics.

LIST OF ABBREVIATIONS AND ACRONYMS

ADH	**alcohol dehydrogenase**
CSAF	**chemical-specific adjustment factor**
CYP	**cytochrome P-450**
E	**hepatic extraction ratio in average adult**
E_{hep}	**hepatic extraction ratio**
E_{ren}	**renal extraction ratio**
GFR	**glomerular filtration rate**
GSD	**geometric standard deviation**
HKAF	**human kinetic adjustment factor**
Km	**Michaelis-Menten constant**
MSP	**microsomal protein**
Pb	**blood:air partition coefficient**
PBPK	**physiologically-based pharmacokinetic**
Qc	**cardiac output**
Qk	**kidney blood flow**
Ql	**liver blood flow**
Qp	**alveolar ventilation rate**
RAM	**rate of metabolism**
RfC	**reference concentration**
RfD	**reference dose**
SD	**standard deviation**
Vmax	**maximum rate of metabolism**
Vl	**volume of liver**
VOC	**volatile organic compound**

4.1 Introduction

A default uncertainty factor of 10 is usually applied to account for human interindividual variability in non-cancer risk assessments (Dourson *et al.*, 1996; Dourson and Stara, 1983; U.S. EPA, 2002). It has been proposed that the variability in the toxicokinetics and toxicodynamics of a chemical can be considered separately and that both components be given a default value of 3.16 (√10) (Dorne and Renwick 2005; IPCS, 1994). The adequacy of this default value for specific chemicals can be assessed and replaced, as appropriate, by quantifying chemical-specific adjustment factors (CSAFs), as proposed by the International Programme on Chemical Safety (IPCS, 2005; Meek *et al.*, 2002). Using this approach, the CSAF for interindividual variability in toxicokinetics, also referred to as the human kinetic adjustment factor (HKAF), can be determined based on distributions of pharmacokinetic parameters (e.g., half-life and maximal concentration) in the studied population (IPCS, 2005; Meek *et al.*, 2002). The magnitude of the HKAF for oral exposures has been inferred by analyzing a therapeutic drug database. Precisely, metabolic pathway-related differences were analyzed based on pharmacokinetic parameters among healthy adults with different phenotypes and other subpopulations, following oral or intravenous administration of pathway-specific probe substrates (Dorne *et al.*, 2005; Ginsberg *et al.*, 2002). Data for pharmaceutical substances have been evaluated as a function of clearance pathways, such as glucuronidation (Dorne *et al.*, 2001a), renal function (Dorne *et al.*, 2004b) or hepatic metabolism by CYP (*i.e.*, 1A2 (Dorne *et al.*, 2001b), 2C19 (Dorne *et al.*, 2003b), 2D6 (Dorne *et al.*, 2002), and 3A4 (Dorne *et al.*, 2003a)). However, HKAF is minimally developed for other pathways and substances with physicochemical characteristics of relevance to environmental toxicants (Dorne *et al.*, 2004a) because the data are sparse or inappropriate to facilitate such an analysis. Among these pathways figure CYP2E1, CYP1A1 and alcohol dehydrogenase (ADH). CYP2E1 is involved in the metabolism of low molecular weight halogenated hydrocarbons, such as carbon tetrachloride, trichloroethylene, and vinyl chloride (Ronis *et al.* 1996), whereas ADH biotransforms water soluble compounds, such as isopropanol, 2-methoxy and 2-butoxy ethanol (Clewell *et al.*

2001; Corley *et al.*, 1994, 2005), and CYP1A1 metabolizes PAHs and dioxin (Kawajiri and Hayashi, 1996).

Since data on the kinetics of environmental toxicants in sensitive subpopulations are unavailable due to ethical considerations, physiologically based pharmacokinetic (PBPK) models (Clewell *et al.*, 2004; Gentry *et al.*, 2002; Nong *et al.*, 2006; Pelekis *et al.*, 2001, 2003) and steady-state algorithms (Nong and Krishnan, 2007; Pelekis *et al.*, 2001) have been used to explore the adequacy of HKAF with regard to these toxicants. Besides, IPCS (2005) has developed HKAFs for several hypothetical chemicals using a steady-state algorithm (AUC = dose/clearance) for the oral route. In this process, the oral bioavailability was assumed to correspond to 100 %. Therefore, the resulting HKAFs reflected interindividual variability in systemic clearance. The magnitude of the HKAF as reflected by population variability in total systemic clearance (inhalation, hepatic and renal), with specific regard to the metabolic pathways and physico-chemical characteristics of relevance to environmental toxicants, has not been investigated. The objective of this study was thus to evaluate the influence of physico-chemical and biochemical characteristics impacting the systemic clearance on the magnitude of HKAF for chemicals metabolized by hepatic CYP2E1, CYP1A2 (1A1), CYP3A4 and ADH, in selected subpopulations (*i.e.*, adults, neonates, elderly, pregnant women).

4.2 Methods

The overall approach involved using a physiologically based steady-state algorithm to compute the HKAF based on distributions of internal dose metrics for CYP2E1, 1A2 (1A1), 3A4 and ADH substrates in various subpopulations (*i.e.*, adults, neonates, elderly, pregnant women) by means of Monte Carlo simulations. Precisely, HKAF was computed as [95^{th} percentile in subpopulation/median in adults] for hypothetical chemicals exhibiting various hepatic extraction ratios and blood:air partition coefficient (Pb).

4.2.1 Steady-state algorithm

For continuous, lifetime exposures leading to steady-state conditions, the amount of chemical entering the body equals the amount cleared from the body. In such cases, some internal dose metrics (e.g., arterial blood concentration (C_{blood}) and rate of metabolism (RAM)) are essentially constant and can be calculated using a steady-state pharmacokinetic algorithm (Andersen, 1981; Nong *et al.* 2006; Pelekis *et al.*, 1997). Thus, for each subpopulation studied, C_{blood} was computed as a function of the exposure dose rate and the systemic clearance, with the latter being the sum of hepatic ($Ql \times E_{hep}$), pulmonary (Qp/Pb) and renal ($Qk \times E_{ren}$) clearances (Winter, 2010):

$$C_{blood} = \frac{DOSErate}{Ql \times E_{hep} + Qp/Pb + Qk \times E_{ren}} \quad (1)$$

In Eq. 1, Qp is the alveolar ventilation rate, Ql and Qk are the liver and kidney blood flows, respectively, whereas E_{hep} and E_{ren} are the hepatic and renal extraction ratios of the chemical, respectively. The above equation was applied for i) inhalation exposure and ii) body weight- adjusted systemic exposure (hereafter referred to as "systemic exposure"). Therefore, DOSErate corresponds to Qp × the concentration in air for inhalation exposure (Pelekis *et al.*, 1997), and to the dose (mg/kg-d) times the body weight for the systemic exposure (IPCS, 2005). E_{hep} was based on liver blood flow and the intrinsic clearance of a chemical (Clint) (Gibaldi and Perrier, 1982):

$$E_{hep} = \frac{Clint}{Clint + Ql} \quad (2)$$

Based on the same physiological approach as for E_{hep} (Gibaldi and Perrier, 1982; Krishnan *et al.* 2010), E_{ren} was computed as the ratio of the glomerular filtration rate (GFR) on the sum of GFR and the kidney blood flow. However, the possibility of active secretion and reabsorption was not considered in the modeling:

$$E_{ren} = \frac{GFR}{GFR + Qk} \tag{3}$$

Finally, liver volume (Vl)-adjusted RAM was computed from C_{blood} and the hepatic clearance (Price *et al.*, 2003a):

$$RAM = \frac{C_{blood} \times Ql \times E}{Vl} \tag{4}$$

4.2.2 Input parameters and studied populations

4.2.2.1 Independent parameters

The steady-state algorithm was developed for adults and three frequently identified or presumed sensitive subpopulations: neonates (ages 0–30 days), elderly (ages 65–90 years) and pregnant women (age 29, 0–40 weeks pregnant). The distributions for the required parameters used in the steady-state algorithm were taken from the literature (Table 4-I). The body weight and body height data were obtained from the P^3M software (The lifeline Group, Annandale, VA) for 2000 adults and the elderly of both genders (1000 each). For pregnant women, P^3M data for 1,000 non-pregnant women (ages 14–44 years) were used along with a mean body weight increase (BWinc) during pregnancy. The distribution for BWinc was defined considering the specific BWinc at each pregnancy week, obtained with the following equation based on fitting to the ICRP (2002) data at pregnancy weeks 10, 20, 30 and 38:

$$BWinc = 0.0076w^2 + 0.0525w - 0.1945 \quad (5)$$

For neonates, body weight and body height data were taken from Johnsrud *et al.* (2003) and Nelson (1991). All distributions were assumed to be lognormal, based on Thomas *et al.* (1996), except for BWinc (assumed normal).

The metabolic pathways investigated in the current study were those relevant to environmental toxicants (see Section 4.1) and for which data on enzyme ontogeny were available. Distributions describing CYP2E1 ontogeny were taken from Johnsrud *et al.* (2003) for neonates and from Lipscomb *et al.* (2003) for the other subpopulations. For subpopulations other than neonates, distributions describing CYP1A2 (a proxy for the CYP1A1 pathway) and CYP3A4 enzyme content were taken from Shimada *et al.* (1994). The distributions for neonates were estimated based on their relative activity, as compared to adults, reported by Sonnier and Cresteil (1998) for CYP1A2 and by Lacroix *et al.* (1997) for CYP3A4. Means were adjusted according to the ratios of activity, and the standard deviations were set to correspond to the same coefficient of variation as in adults. Mean values for ADH activity in each subpopulation were based on an interpolation of data, described by Sarangapani *et al.* (2003). However, due to the lack of data, the related coefficients of variation were computed as the mean of the coefficients of variations used for the three CYP pathways.

GFR data in adults and the elderly were computed by interpolating GFR at each age, assuming a peak value of 127 ml/min-1.73m^2 at age 30 and an annual decrease of 0.67 % thereafter (Sarangapani *et al.*, 2003). In pregnant women, an increase of 30 % was described, as compared to non-pregnant women (Faustman and Ribeiro, 1990). The

distributions for GFR in neonates were estimated from the data sources identified by DeWoskin and Thompson (2008).

4.2.2.2 Calculated parameters

For the purpose of the present study, physiological parameters were computed from body weight (BW) and body height (BH) data using the average of gender-specific equations of Price *et al.* (2003b), for each subpopulation:

$$\text{Qp (L/min)} = \frac{(0.2519 \times \text{BW}^{0.7609}) + (0.2508 \times \text{BW}^{0.7815})}{2} \quad (6)$$

$$\text{Vl (L)} = 0.05012 \times \text{BW}^{0.78} \quad (7)$$

$$\text{Ql (L/min)} = 0.92 \times \text{Vl} \quad (8)$$

$$\text{Qk (L/min)} = \frac{(3.45 \times ((4.214 \times \text{BW}^{0.823}) + (4.456 \times \text{BW}^{0.795}))}{1{,}000} \quad (9)$$

Since the GFR data were normalized to the body surface area (BSA), absolute GFR values were obtained using the body surface (BSA) (Price *et al.*, 2003b):

$$\text{BSA (m}^2\text{)} = \frac{\text{BW}^{0.515} \times \text{BH}^{0.422} \times 234.9}{10{,}000} \quad (10)$$

For pregnant women, the Dubois and Dubois equation was used to determine the body surface area, as validated by Wang *et al.* (1992). The blood flow to the kidneys in adults was increased by 15 % in pregnant women, as per Faustman and Ribeiro (1990); Qp and Ql were computed using the body weight of non-pregnant women-specific equations, where

Qp (L/min) = (0.2508 × $BW^{0.7815}$) and Ql (L/min) = Vl (L) (Price *et al.*, 2003b). Qp was then multiplied by the mean increase in cardiac output (Qc) at any week of pregnancy (w), so that Qp = Qc. The distribution for this increase (28 ± 9 %, 2–54 %) was estimated by an empirical equation obtained from fitting the ICRP (2002) data on cardiac output for weeks (w) 10, 20, 30 and 38:

$$Qc = 0.0001w^3 - 0.0086w^2 + 0.2203w + 5.0092 \quad (11)$$

Finally, given the possible variability in the physiology of two individuals of identical age and body mass index, a "variability term" was added as a multiplier to the results of Eqs. 6–9. These terms are characterized by the distributions indicated in Table 4-I and are based on data detailed previously (Valcke and Krishnan, 2011) as well as on Thomas *et al.* (1996).

4.2.3 Evaluation of the steady-state algorithm

The capability of the steady-state algorithm to reproduce experimental data for substrates of the metabolic enzymes/pathways investigated in the current study was evaluated by introducing the body weight (and height when available) of the subjects involved in the experimental studies, along with corresponding exposure doses, into the algorithm (*i.e.*, Eq. 1). The resulting C_{blood} values were then compared with those measured during the studies. When these consisted of venous blood data, the C_{blood} values calculated were corrected as per Pelekis *et al.* (1997). Thus, experiments with VOCs, theophylline, fentanyl and alfentanil were considered to evaluate the algorithm to predict interindividual variability related to the metabolic clearance mediated by CYP2E1 and ADH, CYP1A2, and CYP3A4 pathways, respectively. The adequacy of the algorithm with regard to its capacity to account for filtration-mediated renal clearance was assessed based on data from studies using antibiotics that are principally cleared by renal filtration (*i.e.*, ceftizoxime, vancomycin and gentamicin) (Dorne *et al.*, 2004b). During this particular evaluation

process however, the pulmonary clearance of antibiotics was considered insignificant by attributing a very high value of Pb (1,000,000). Because these drugs are significantly bound to plasma proteins, the hepatic and renal clearances in Eq. 1 were also multiplied by the unbound fraction of drugs in blood, calculated as per Johnson *et al.* (2006) using the relative protein content described by Ehrnebo *et al.* (1971) for adults and neonates,. The fraction unbound for theophylline and fentanyl/alfentanil were respectively 44 % (Rosen *et al.*, 1979) and 16 % (Bjorkman and Redke, 2000). For antibiotics, corresponding values were respectively 60 %, 50 % and 83 % for ceftizoxime, vancomycin and gentamicin (Borin *et al.*, 1990; Kusama *et al.*, 1998; Landers *et al.*, 1984).

Experimental data in adults for trichloroethylene, 1,1,1-trichloroethane, toluene and m-xylene (Laparé *et al.* 1993, 1995; Veulemans and Masschelein, 1978) were reproduced using the steady-state algorithm. For experimental studies involving drugs, pharmacokinetic data for theophylline were taken from Ginsberg *et al.* (2004), and data for fentanyl were taken from Bjorkman (2003). Fentanyl data were also used for alfentanil due to data gaps. Ginsberg *et al.* (2004) expressed the intrinsic clearance of theophylline as a function of the molar amount of each of its metabolites formed per hour and per mol of CYP1A2 (*i.e.*, 1-methylxanthine, 3-methylxanthine and dimethyl uric acid). Since these metabolites directly result from theophylline (Ginsberg *et al.*, 2004), the total intrinsic clearance of theophylline was assumed to correspond to the sum of the three metabolite-related clearances (*i.e.*, 2.992×10^6 L/h-mol of CYP1A2). Similarly, hepatic clearance of fentanyl was expressed as 766 ml/min-kg of liver in adults by Bjorkman (2003). Since these two clearance values were determined in adults, the hepatic clearance in each subpopulation was calculated based on the total amount of CYPs in the liver and its volume, while taking into account the microsomal content of CYP1A2 and CYP3A4 as described in Table 4-I. In the process, a microsomal protein hepatic content of 32 mg/g of liver (Lipscomb and Poet, 2008) was used and assumed to be comparable across subpopulations, given the lack of data suggesting otherwise.

The steady-state algorithm was then evaluated using steady-state experimental studies in adults and non-adults, as described by various authors for theophylline (Bachmann *et al.*, 1990, 1993; Davy *et al.*, 1999; Giacoia *et al.*, 1976; Gonzalez *et al.*, 1994; Gotz *et al.*, 1994; Jonkman *et al.*, 1991; Reinhardt *et al.*, 1987; Simons and Simons, 1978; Vincent *et al.*, 1997) and fentanyl/alfentanil (Meistelman *et al.*, 1987; Saarenmaa *et al.*, 2000; Santeiro *et al.*, 1997). The hepatic clearance was set to 0 for the renally-cleared chemicals (antibiotics) while comparing the steady-state algorithm results with data obtained in adults and children (Asbury *et al.*, 1993; Borin *et al.*, 1990; Healy *et al.*, 1987; Kildoo *et al.*, 1989 Landers *et al.*, 1984).

4.2.4 Monte Carlo simulations and HKAF matrices

For a low, level of exposure (typically well below metabolic saturation) corresponding to a systemic exposure rate of 10 µg/kg-d and an inhalation exposure to 10 mg/m^3, Monte Carlo simulations were performed using the Crystal Ball® software (Oracle™, Redwood Shores, CA) to generate distributions for C_{blood} and RAM on the basis of Eqs 1 to 4. In the process, 5000 iterations were realized for each subpopulation. To avoid unrealistic combinations, body weight and height were correlated to 60 % based on the body mass index population distribution in Canada (Statistics Canada, 2003). These simulations were performed for the four pathways studied, combining each of nine Pb values (1, 10, 20, 50, 100, 300, 1000, 3000 and 10,000) with each of nine values for the hepatic extraction ratio in an average 70 kg adult with mean enzyme content of Table 4-I (referred to as "E" for the rest of this article). The nine E values considered *i.e.* 0.01, 0.1, 0.2, 0.3, 0.5, 0.7, 0.8, 0.9, and 0.99, covered the range of hepatic extraction ratios from 1 % to 99 % to reflect the variability between "poorly metabolized" and "highly metabolized" chemicals. For each of these values, the intrinsic clearance was determined according to Eq. 2, for a liver blood flow of 76 L/h, as determined by applying Eqs. 7 and 8 to the average adult (Table 4-II). For each Monte Carlo iteration, the intrinsic clearance for a given individual in any subpopulation

($Clint_{subpop}$) was calculated based on the pathway-specific intrinsic clearance in the average adult ($Clint_{ad}$, see Table 4-II) adjusted for the liver volume and enzyme content, as per Eq. 13 below, modified from Nong *et al.* (2006):

$$Clint_{subpop} = Clint_{ad} \times Vl_{subpop} \times [enzyme]_{subpop} \quad (13)$$

The resulting distributions for C_{blood} and RAM were used in the calculation of the HKAF, *i.e.*, for computing the ratio of the 95th percentile value in the subpopulations to the median value (50th percentile) in adults (IPCS, 2005). For each combination of E and Pb, the highest HKAF value from those obtained for the various subpopulations was then reported in a route-, pathway-, and dose metric-specific 9×9 matrix (Fig. 4.1).

4.2.5 Sensitivity analyses

The most influential parameters of the algorithm in each subpopulation were determined by sensitivity analyses. For a parameter P, sensitivity index SI, based on dose metrics DM, was:

$$SI = \frac{DM_{10} - DM_i}{P_{10} - P_i} \times \frac{P_i}{DM_i} \quad (14)$$

In this equation, subscript 10 denotes the dose surrogate and parameter value when the parameter value is reduced by 10 % compared to the initial value (subscript i). The greater the absolute value of SI, the greater the influence (positive or negative) on the output. For example, if changing the input value by 10 % resulted in a 6 % increase, the resulting sensitivity index would be 0.6. The sensitivity analyses were performed for six clearance-

related scenarios defined for presumably sensitive neonates, on the basis of relevant terms in Eq. 1 (Table 4-I):

A. Pulmonary clearance / hepatic clearance > 100 (E = 0.01, Pb = 10);

B. Pulmonary clearance ≈ perfusion-limited hepatic clearance (E = 0.8, Pb = 10);

C. Pulmonary clearance ≈ enzyme-limited hepatic clearance (E = 0.2, Pb = 50);

D. Pulmonary clearance / perfusion-limited hepatic clearance < 0.01 (E = 0.9, Pb = 3000);

E. Pulmonary clearance / enzyme-limited hepatic clearance < 0.01 (E = 0.2, Pb = 3000);

F. Renal clearance only (E = 0.01, Pb = 10,000).

Additionally, the impact of enzyme content on C_{blood} when the pulmonary clearance is reduced (Pb ≥ 100) was further analyzed for a range of E values corresponding to enzyme-limited metabolism, *i.e.* when ≤ 0.5.

4.3 Results

4.3.1 Evaluation of the steady-state algorithm

The C_{blood} values obtained using the steady-state algorithm were within a two-fold factor of the mean from the published experimental values in 30 out of the 34 cases evaluated (Fig. 4.2). These include 12 out of 13 predictions for VOCs, 8 out of 10 predictions for theophylline, 3 out of 4 predictions for fentanyl/alfentanil and 7 out of 7 predictions for the antibiotics. These findings are consistent with the fact that the steady-state C_{blood} computation (Eq. 1) adequately reflects continuous inhalation exposure to VOCs, despite

the fact that respiration is actually cyclic, leading to complex absorption/desorption behaviour in the airways, and thus not as simple as modeled; and (2) is within the range of the maximum and trough steady-state concentrations typical of sequential intakes in experimental studies with drugs.

4.3.2 HKAF matrices for systemic and inhalation exposures

The range of dose metrics obtained in the average individual of each subpopulation for continuous exposures to CYP2E1 and CYP1A2 substrates are detailed in appendices A and B. The C_{blood}-based HKAF matrices for systemic exposure are shown in Fig. 4.3. Exceedance of the 3.16 default factor was observed for CYP1A2 substrates in neonates only in some cases (max: 6.1, Fig. 4.3). These exceedances occurred when the ratio of the body weight-adjusted systemic clearance in the average adult to the average neonate is at least equal to 2.2 (Fig. 4.4). In all other cases (subpopulations and pathways), the C_{blood}-based HKAF remained below 3.16 (Fig. 4.3, Table 4-III). Neonates consistently exhibited the highest HKAF (*i.e.* referred hereafter to "the most sensitive subpopulation", based on pharmacokinetic considerations) for chemicals with high Pbs, and E values up to 0.9 for the CYP1A2 pathway and up to 0.7 or 0.8 for the other pathways (Fig. 4.3). Adults were the most sensitive subpopulation for chemicals with very low Pb values (≤ 10). Elderly were the most sensitive in the other cases.

For inhalation exposure (Fig. 4.5), greater C_{blood}-based HKAFs were observed in neonates for most pathways and combinations of E and Pb values. However, pregnant women showed greatest HKAF when the CYP2E1, CYP3A4, and ADH pathways exhibit E = 0.99. Default value exceedances were observed in neonates only (Table 4-III), reaching 5.4 and 12.4 for the CYP2E1 and CYP1A2 pathways, respectively. For the CYP2E1 pathway, the default value of 3.16 was exceeded when the following occurred: (1) E = 0.01 and Pb ≥ 1000; (2) E = 0.1–0.2 and Pb ≥ 300; (3) E = 0.3–0.7 and Pb ≥ 100. Similar trends are

observed for CYP3A4 and ADH pathway, whereas for CYP1A2 pathway, values of HKAF > 3.16 are observed when E gets as high as 0.9 along with Pb ≥ 50. Also, HKAF ≥ 10 were obtained for E values of 0.3–0.7 along with high Pb values. Contrary to systemic exposure, no trend in the magnitude of the adult-to-neonate ratio of body weight - adjusted clearance was observed.

The RAM-based HKAF never exceeded the default value and did not vary much (Table 4-III), thus no particular trends in matrices were noted (data not shown). Maximum HKAF varied between 1.4 and 2.4. The most sensitive subpopulation for the metabolite dose metrics was generally the elderly for an identical systemic exposure except for very high E and Pb values where pregnant women showed greater HKAFs. For inhalation, pregnant women were more sensitive than other subpopulations except for E = 0.01 (for which elderly were).

4.3.3 Sensitivity analyses

The sensitivity analyses shown in Fig. 4.6 for the systemic exposure demonstrate that when pulmonary clearance drives the systemic clearance (A), Qp is the most influential physiological parameter. When pulmonary and hepatic clearances are similar, Qp shares this influence with Ql and Vl, when hepatic metabolism is perfusion-limited (B). The enzyme content also becomes significant at the expense of Ql, for enzyme-limited metabolism (C, E). Regardless of the metabolic pathway, highest sensitivity for enzyme content was systematically observed in the E range of 0.1–0.3 when Pb is 100 or greater, in both adults and neonates but with consistently greater values in neonates (Fig. 4.7). This range of E and Pb corresponds to the highest HKAF values (Figs. 4.3 and 4.5). The sensitivity for Qp in neonates is greater as compared to the other subpopulations in scenarios B and C. This is also the case for CYP content for perfusion-limited metabolism

(B, D), which drives the systemic clearance in scenario D. In such cases, Qp is not as influential (nor is enzyme content), but hepatic parameters (Ql, Vl) are, regardless of the subpopulation. Finally, when renal clearance is the main elimination pathway (F), GFR and parameters related to body surface area (body weight and body height) are the most influential parameters in every subpopulation. However, Qk is not because Qk >>> GFR, and thus, the renal clearance is "GFR-limited". GFR (and body surface area) is also somewhat influential when the sum of the hepatic and pulmonary clearances is low (C, E). Body weight always exhibits a relatively high influence on C_{blood} output given that it is used to compute every physiological parameter in the steady-state algorithm. Similar patterns were observed on the basis of RAM (data not shown), but the impact of CYP and Ql on RAM was reversed as compared to their impact on C_{blood}.

4.4 Discussion

The present work applied a probabilistic method to a steady-state algorithm to evaluate the magnitude of the HKAF as a function of physico- and bio-chemical characteristics impacting the systemic clearance of chemicals. We used the steady-state algorithm because of the chronic exposures, which are relevant to regulatory guideline development, and the very high number of combinations explored (*i.e.*, metabolic pathways, exposure route, hepatic extraction ratios, Pb values, and subpopulations). Such algorithm, while requiring fewer input parameters, has been shown to provide identical results as full blown PBPK models for a variety of inhaled VOCs (Andersen, 1981; Pelekis *et al.*, 1997, 2001). Overall, the exercises conducted suggest that the default HKAF may be exceeded in neonates when a particular combination is obtained, *i.e.* when the toxic moiety considered is the parental form, the pulmonary clearance is low or negligible and the hepatic metabolism is not perfusion-limited. Besides, for the systemic exposure, the adult/neonate ratio of body weight-adjusted systemic clearance needs to reach at least 2.2, which was observed in some cases involving CYP1A2 substrates (Figs. 4.3–4.4).

The HKAF values obtained appear reasonable in view of results obtained in other studies. Indeed, Dorne *et al.* (2001b) obtained adult/neonate ratios for mean clearances varying between 2.9 (theophylline) and 13.9 (caffeine) for oral and IV exposures. Ginsberg *et al.* (2002) reported neonate/adult ratios for half-lives varying between 4.5 and 17 for these same drugs. For CYP3A4 substrates, HKAF values ranging from 2.3 to 4 were obtained by Dorne *et al.* (2003a) based on mean clearance values. Mean half-life ratios of less than 2 were observed for CYP 3A substrates by Ginsberg *et al.* (2002). For CYP2E1 and ADH substrates, data on adult/child differences are limited, but Pelekis *et al.* (2003) obtained a ratio of 95th percentile at age 1 *vs* median at age 30 of venous blood concentration of inhaled dichloromethane slightly above 2, considering continuous lifetime exposure. Besides, Nong *et al.* (2006) obtained HKAF values varying between 2.5 and 3.9 for inhaled toluene, a highly metabolized CYP2E1 substrate with a Pb value of 15.6. Clewell *et al.* (2004) obtained a child/adult ratio varying between 0.3 and 1.4 for oral exposure to three CYP2E1-metabolized VOCs with Pb < 20 and of 2 for inhaled isopropanol (Pb = 838), an extensively metabolized ADH substrate. Finally, Ginsberg *et al.* (2005) obtained child/adult ratios varying between 1 and 1.7 on the basis of steady-state blood concentration of inhaled CYP2E1 substrates. The ratios increased with the increasing Pb from 1 to 50, and the decreasing fraction of hepatic clearance relative to hepatic blood flow from 100 to 0.3 Corresponding ratios for CYP1A2 substrates were 1–2.7. All those results appear rather comparable to the route- and pathway-specific HKAF obtained herein for corresponding E and Pb values. This is interesting in view of the fact that the other studies have mainly evaluated CYP1A2 and 3A4 substrates' pharmacokinetic parameters (e.g., clearances or half-lives) and not their internal dose (Dorne *et al.* 2001b, 2003; Ginsberg *et al.* 2002), considered non-steady-state exposure (Nong *et al.*, 2006), or applied a deterministic (Clewell *et al.* 2004; Ginsberg *et al.* 2005) or only partly probabilistic approach (Pelekis *et al.* 2003). It is also noteworthy that for systemic exposure, the HKAF values obtained in neonates (2.0–2.3) when Pb = 10,000 and E = 0.01, *i.e.* when renal clearance contributed to near 90 % of total systemic clearance (Fig. 4.8), were comparable to the HKAF value determined for boron (2.0) on the basis of the variability in GFR in another subpopulation

(pregnant women) (U.S. EPA, 2004). These HKAF values were also slightly lower than the neonate/adult ratio (2.8) of the geometric means of half-lives for renally-cleared chemicals reported by Ginsberg *et al.* (2002) and Dorne *et al.* (2004b). Overall then, the findings of our study present the specific case of chronic exposures to environmental contaminants accounting for both inter- and intra-subpopulation variability in toxicokinetics on one hand, while being supported by the results of previous studies on the other hand.

Small variations between the HKAFs obtained in each subpopulation for every combination of E and Pb can be inherent to the Monte Carlo simulation process, but stronger trends are likely related to true differences in the physiological determinants of pharmacokinetics. The high C_{blood}-based HKAFs for the inhalation route in neonates (and pregnant women) are explained by high intake due to greater-than-adult body weight-adjusted ventilation rate (Faustman and Ribeiro, 1990; Valcke and Krishnan, 2009). A combination of factors explains the results for systemic exposure. Dosimetrically, adults were the most sensitive among all the subpopulations evaluated for chemicals with lower Pb (*i.e.*, Pb ≈ 1) because they were exposed to a greater absolute dose than the neonates and their pulmonary clearance was lower on a body weight basis. With increasing Pb however (Pb = 10–50), the relative contribution of pulmonary clearance to systemic clearance would be reduced. As a result, the contribution of renal clearance increased proportionally, making the elderly more sensitive. For chemicals that are not extensively metabolized however (E < ≈ 0.9), further increase of the Pb value (≈ 50) resulted in the neonates becoming more sensitive due to the combined effect of negligible pulmonary clearance and enzyme-limited metabolism (Johnsrud *et al.*, 2003; Lacroix *et al.*, 1997; Sarangapani *et al.*, 2003; Sonnier and Cresteil, 1998). This phenomenon was not observed at very high E values when metabolism is perfusion-limited. Lower neonate-to-adult ratio of CYP1A2 enzyme as compared to other enzymes (2 % *vs* 25–35 %, Table 4-I) resulted in greater HKAF values for CYP1A2 pathway. On the basis of RAM (data not shown), greater HKAF values were observed in the subpopulations exhibiting fully expressed hepatic enzymes along with the greatest blood concentration of parent compound in order to biotransform it into metabolites, *i.e.*

elderly or pregnant women (see Results). All these considerations point out the importance of determining the impact of uncertainty associated with the key determinants (identified in the sensitivity analyses, Fig. 4.6), on the estimation of population variability in pharmacokinetics and the resulting HKAF for various combinations of E and Pb. For instance, reducing the uncertainty on the enzyme content/activity appears particularly important when metabolism is enzyme-limited, *i.e.* when E = 0.1–0.3 (Fig. 4.7), mostly in neonates for whom lower enzyme content compared to adults (Fig. 4.7A) leads to high HKAF values (Figs 4.3 and 4.5).

Although the consideration of renal clearance is innovative as compared to previously published steady-state models (Andersen, 1981; Csanàdy and Filser, 2001; Nong and Krishnan, 2007; Pelekis *et al.*, 2001), assuming that it can be approximated using only GFR and Qk constitutes a limitation to the present study. While this assumption is reasonable for some filtered chemicals (Fig. 4.2c), it may not be so for other chemicals. At this time, however, generic rates of active secretion and tubular reabsorption can not be computed for surrogate environmental chemicals, as they are likely to be chemical-specific. Qk might account for part of the variability in active secretion as proposed by Clewell *et al.* (2004) for the renal clearance of trichloroacetic acid. Reabsorption appears more relevant to liposoluble VOCs that are also often quite volatile and highly cleared by exhalation (Pelekis and Krishnan, 2004). Thus, the impact of neglecting the reabsorption may have been minimal in our study since this process is likely more associated with chemicals that are thoroughly cleared by pulmonary clearance anyways, due to low Pb. For chemicals with high Pb, greater water solubility makes them unlikely candidates for tubular reabsorption, and the consideration of filtration-mediated renal clearance appears reasonable. The evaluation of the steady-state algorithm for inhaled VOCs (Fig. 4.2a) supports this reasoning. Besides, Fig. 4.8 shows that the inclusion of the renal clearance term as defined in the present study had a generally low impact on the overall systemic clearance in adults for chemicals that are highly metabolized ($E \geq 0.7$), regardless of the Pb considered. Very

similar results were obtained in other subpopulations (data not shown). For partly or poorly metabolized chemicals ($E \leq 0.5$), renal clearance may be considered negligible (*i.e.* it contributes to 10 % or less of the systemic clearance) when Pb is below 50. Results of the sensitivity analyses described above (Fig 4.6c. e and f) also follow these observations. The impact of the consideration of renal clearance on the different HKAF would likely depend of the differences between each subpopulation with regards to the relative contribution of the renal clearance as compared to the overall systemic clearance. Obviously, in the case where renal clearance would equal kidney blood flow, its contribution to the systemic clearance would be higher, *i.e.* comparable to that of liver clearance given similar tissue blood flows (see Eq. 8-9). Because on a body weight-basis, kidney blood flow, contrary to GFR, is greater in neonates than adults (Valcke and Krishnan, 2011), this would lead to a possible reduction of the projected HKAF.

Another limitation in the current study relates to the fact that among the input parameters of the steady-state algorithm, the Pb was treated as being age-invariant. In this regard, Malviya and Lerman (1990) reported that Pb varied by, at the most, 14 % between neonates and adults. This variation appears insignificant in view of the range of Pb values considered in this study, and is consistent with previous efforts in the literature (Clewell *et al.*, 2004; Valcke and Krishnan, 2009). Also, the liver blood flow used in this study to derive E values (76 L/h, Table 4-II) corresponds to a slightly lower percentage of Qc (21 %, based on $Qc = 15\ L/h\text{-}kg^{0.75}$, for a 70 kg adult) than the 25 % often used in PBPK modeling. However, this value results from an equation (Price *et al.* 2003b) which was applied to every subpopulation and is within the range of values reported in the literature (e.g. Brown *et al.*, 1997; Thomas *et al.*, 1996). Finally, this study focused on characterizing the magnitude of the interindividual variability in kinetics due to the systemic clearance, for a low inhalation concentration or systemically-available absorbed dose, similar to the case studies developed by IPCS (2005). Further studies on the potential impact of the interindividual variability in other toxicokinetic determinants such as the oral bioavailability or the plasma protein binding are needed to extend these findings.

The matrices developed here represent potential tools for developing a framework for estimating the HKAF for inhalation or systemic exposures when human data are sparse for a given substance, along the principle of establishing "categorical adjustment factors" (Naumann *et al.* 2001). The only required data are E, which can be estimated from animal or *in vitro* data, and Pb, which can be evaluated based on physico-chemical characteristics (Poulin and Krishnan, 1996). Fig. 4.3 may also be useful to determine whether or not a GFR-mediated renal clearance term should be considered in the process. The approach developed in this study can be expanded to evaluate default factor to adequately cover other subpopulations (e.g., obese, patients), to evaluate the impact of alternative dose metrics including metabolite dose (*i.e.*, metabolite concentration in target tissues) and to assess the impact of the consideration of various percentile values (e.g., 99 *vs* 95) on the resulting HKAF.

In conclusion, the present work has brought out critical differences in the HKAF value relevant to environmental contaminants metabolized by specific isozymes (*i.e.*, CYP2E1, CYP1A2, CYP3A4 and ADH). It has also pointed out the importance of the internal dose surrogate, Pb and E for the identification of dosimetrically most sensitive subpopulation and the HKAF value associated with each one of them. This study allowed identifying cases were the adequacy of the default HKAF should be verified, particularly with regard to the protection of neonates, the only subpopulation in which the default HKAF was sometimes exceeded. This information should then be used along with the proportion of this subpopulation in the target population of relevance to a particular risk assessment. Such an approach will ensure that the HKAF value would be protective of all subpopulations of concern for specific risk assessment and management considerations.

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4.5 References

Andersen, M. E. 1981. A physiologically based toxicokinetic description of the metabolism of inhaled gases and vapors: analysis at steady state. *Toxicol Appl. Pharmacol. 60*, 509-526.

Asbury, W. H., Darsey, E. H., Rose, W. B., Murphy, J. E., Buffington, D. E., Capers, C. C. 1993. Vancomycin pharmacokinetics in neonates and infants: a retrospective evaluation. *Ann. Pharmacother. 27*, 490-496.

Bachmann, K., Jauregui, L., Sides, G., Sullivan, T. J. 1993. Steady-state pharmacokinetics of theophylline in COPD patients treated with dirithromycin. *J Clin. Pharmacol. 33*, 861-865.

Bachmann, K., Nunlee, M., Martin, M., Sullivan, T., Jauregui, L., DeSante, K., Sides, G. D. 1990. Changes in the steady-state pharmacokinetics of theophylline during treatment with dirithromycin. *J Clin. Pharmacol. 30*, 1001-1005.

Bjorkman, S. 2003. Reduction and lumping of physiologically based pharmacokinetic models: prediction of the disposition of fentanyl and pethidine in humans by successively simplified models. *J Pharmacokinet. Pharmacodyn. 30*, 285-307.

Bjorkman, S., Redke, F. 2000. Clearance of fentanyl, alfentanil, methohexitone, thiopentone and ketamine in relation to estimated hepatic blood flow in several animal species: application to prediction of clearance in man. *J Pharm. Pharmacol.* 52, 1065-1074.

Borin, M. T., Hughes, G. S., Spillers, C. R., Patel, R. K. 1990. Pharmacokinetics of cefpodoxime in plasma and skin blister fluid following oral dosing of cefpodoxime proxetil. *Antimicrob. Agents Chemother. 34*, 1094-1099.

Brown, R. P., Delp, M. D., Lindstedt, S. L., Rhomberg, L. R., Beliles, R. P. 1997. Physiological parameters values for physiologically based pharmacokinetic models. *Toxicol. Ind. Health. 13*, 407-484.

Clewell, H. J., Gentry, P. R., Covington, T. R., Sarangapani, R., Teeguarden, J. G. 2004. Evaluation of the potential impact of age- and gender-specific pharmacokinetic differences on tissue dosimetry. *Toxicol Sci. 79*, 381-393.

Clewell, H. J., III., Gentry, P. R., Gearhart, J. M., Covington, T. R., Banton, M. I., Andersen, M. E. 2001. Development of a physiologically based pharmacokinetic model of isopropanol and its metabolite acetone. *Toxicol Sci. 63*, 160-172.

Corley, R. A., Bartels, M. J., Carney, E. W., Weitz, K. K., Soelberg, J. J., Gies, R. A., Thrall, K. D. 2005. Development of a physiologically based pharmacokinetic model for ethylene glycol and its metabolite, glycolic Acid, in rats and humans. *Toxicol Sci. 85*, 476-490.

Corley, R. A., Bormett, G. A., Ghanayem, B. I. 1994. Physiologically based pharmacokinetics of 2-butoxyethanol and its major metabolite, 2-butoxyacetic acid, in rats and humans. *Toxicol Appl. Pharmacol. 129*, 61-79.

Csanady, G. A., Filser, J. G. 2001. The relevance of physical activity for the kinetics of inhaled gaseous substances. *Arch. Toxicol. 74*, 663-672.

Davy, M., Allen, A., Bird, N., Rost, K. L., Fuder, H. 1999. Lack of effect of gemifloxacin on the steady-state pharmacokinetics of theophylline in healthy volunteers. *Chemotherapy. 45*, 478-484.

DeWoskin, R. S., Thompson, C. M. 2008. Renal clearance parameters for PBPK model analysis of early lifestage differences in the disposition of environmental toxicants. *Regul. Toxicol Pharmacol. 51*, 66-86.

Dorne, J. L., Renwick, A. G. 2005. The refinement of uncertainty/safety factors in risk assessment by the incorporation of data on toxicokinetic variability in humans. *Toxicol. Sci. 86*, 20-26.

Dorne, J. L., Walton, K., Renwick, A.G. 2001a. Human variability in glucuronidation in relation to uncertainty factors for risk assessment. *Food Chem. Toxicol. 39*, 1153-1173.

Dorne, J. L., Walton, K., Renwick, A. G. 2001b. Uncertainty factors for chemical risk assessment. Human variability in the pharmacokinetics of CYP1A2 probe substrates. *Food Chem. Toxicol. 39*, 681-696.

Dorne, J. L., Walton, K., Renwick, A. G. 2003a. Human variability in CYP3A4 metabolism and CYP3A4-related uncertainty factors for risk assessment. *Food Chem. Toxicol. 41*, 201-224.

Dorne, J. L., Walton, K., Renwick, A. G. 2003b. Polymorphic CYP2C19 and N-acetylation: human variability in kinetics and pathway-related uncertainty factors. *Food Chem. Toxicol. 41*, 225-245.

Dorne, J. L., Walton, K., Renwick, A. G. 2004a. Human variability for metabolic pathways with limited data (CYP2A6, CYP2C9, CYP2E1, ADH, esterases, glycine and sulphate conjugation). *Food Chem. Toxicol. 42*, 397-421.

Dorne, J. L., Walton, K., Renwick, A. G. 2004b. Human variability in the renal elimination of foreign compounds and renal excretion-related uncertainty factors for risk assessment. *Food Chem. Toxicol. 42*, 275-298.

Dorne, J. L., Walton, K., Renwick, A. G. 2005. Human variability in xenobiotic metabolism and pathway-related uncertainty factors for chemical risk assessment: a review. *Food Chem. Toxicol. 43*, 203-216.

Dorne, J. L., Walton, K., Slob, W., Renwick, A. G. 2002. Human variability in polymorphic CYP2D6 metabolism: is the kinetic default uncertainty factor adequate? *Food Chem. Toxicol. 40*, 1633-1656.

Dourson, M. L., Felter, S. P., Robinson, D. 1996. Evolution of science-based uncertainty factors in noncancer risk assessment. *Regul. Toxicol. Pharmacol. 24(2 Pt 1)*, 108-120.

Dourson, M. L., Stara, J. F. 1983. Regulatory history and experimental support of uncertainty (safety) factors. *Regul. Toxicol. Pharmacol. 3*, 224-238.

Ehrnebo, M., Agurell, S., Jalling, B., Boreus, L. O. 1971. Age differences in drug binding by plasma proteins: studies on human foetuses, neonates and adults. *Eur. J Clin. Pharmacol. 3*, 189-193.

Faustman, E. M., Ribeiro, P., 1990. Pharmacokinetic consideration in developmental toxicity, IN: Hood, R.D. (Ed.), *Developmental toxicology: risk assessment and the future*. Van Nostrand Reinhold, New York, NY, pp. 109-136.

Gentry, P. R., Hack, C. E., Haber, L., Maier, A., Clewell, H. J., III. 2002. An approach for the quantitative consideration of genetic polymorphism data in chemical risk assessment: examples with warfarin and parathion. *Toxicol. Sci. 70*, 120-139.

Giacoia, G., Jusko, W. J., Menke, J., Koup, J. R. 1976. Theophylline pharmacokinetics in premature infants with apnea. *J Pediatr. 89*, 829-832.

Gibaldi, M., Perrier, D., 1982. Pharmacokinetics, Second ed. Marcel Dekker Inc., New York NY.

Ginsberg, G., Hattis, D., Russ, A., Sonawane, B. 2004. Physiologically based pharmacokinetic (PBPK) modeling of caffeine and theophylline in neonates and adults: implications for assessing children's risks from environmental agents. *J Toxicol. Environ Health A. 67*, 297-329.

Ginsberg, G., Hattis, D., Sonawane, B., Russ, A., Banati, P., Kozlak, M., Smolenski, S., Goble, R. 2002. Evaluation of child/adult pharmacokinetic differences from a database derived from the therapeutic drug literature. *Toxicol. Sci. 66*, 185-200.

Ginsberg, G., Perkovich Foos, B., Firestone, M. P. 2005. Review and analysis of inhalation dosimetry methods for application to children's risk assessment. *J Toxicol. Environ Health A*. 68, 573-615.

Gonzalez, M. A., Kisicki, J., Straughn, A. B. 1994. Pharmacokinetic comparison of a once-daily and twice-daily theophylline delivery system. *Clin. Ther. 16*, 686-692.

Gotz, J., Sauter, R., Steinijans, V. W., Jonkman, J. H. 1994. Steady-state pharmacokinetics of a once-daily theophylline formulation (Euphylong) when given twice daily. *Int. J Clin. Pharmacol Ther. 32*, 168-173.

Healy, D. P., Polk, R. E., Garson, M. L., Rock, D. T., Comstock, T.J. 1987. Comparison of steady-state pharmacokinetics of two dosage regimens of vancomycin in normal volunteers. Antimicrob. *Agents Chemother. 31*, 393-397.

ICRP. 2002. Annals of the ICRP. ICRP Publication 89. *Ann ICRP. 32*, 5-265.

IPCS. 1994. Assessing human health risks of chemicals: Derivation of guidance values for health based exposure limits. 170. Geneva, World Health Organization, International Panel on Chemical Safety. Environmental Health Criteria.

IPCS. 2005. Chemical-Specific Adjustment Factors (CSAFs) for Interspecies Differences and Human Variability: Guidance Document for the Use of Data in Dose/Concentration-Response Assessment. WHO/IPCS/01.4, 1-96. International Panel on Chemical Safety, World Health Organisation, Geneva, Switzerland

Johnson, T. N., Rostami-Hodjegan, A., Tucker, G. T. 2006. Prediction of the clearance of eleven drugs and associated variability in neonates, infants and children. *Clin. Pharmacokinet. 45*, 931-956.

Johnsrud, E. K., Koukouritaki, S. B., Divakaran, K., Brunengraber, L. L., Hines, R. N., McCarver, D. G. 2003. Human hepatic CYP2E1 expression during development. *J Pharmacol. Exp. Ther. 307*, 402-407.

Jonkman, J. H., Sollie, F. A., Sauter, R., Steinijans, V. W. 1991. The influence of caffeine on the steady-state pharmacokinetics of theophylline. *Clin. Pharmacol Ther. 49*, 248-255.

Kawajiri, K., Hayashi, S. I., 1996. The CYP1 family, IN: C. Ioannides (Ed.), *Cytochrome P450 Metabolic and Toxicological Aspects*., CRC Press, Boca Raton FL, pp. 77-88.

Kildoo, C. W., Lin, L. M., Gabriel, M. H., Folli, H. L., Modanlou, H. D. 1989. Vancomycin pharmacokinetics in infants: relationship to postconceptional age and serum creatinine. *Dev. Pharmacol Ther. 14*, 77-83.

Krishnan, K., Loizou, G. D., Spendiff, M., Lipscomb, J. C., Andersen, M. E. 2010. PBPK modeling: a primer, IN: Krishnan, M.E., Andersen, M.E. (Eds.), *Quantitative modeling in toxicology*. John Wiley and Sons Ltd., West Sussex, ENG, pp. 21-58.

Kusama, M., Yamamoto, K., Yamada, H., Kotaki, H., Sato, H., Iga, T. 1998. Effect of cilastatin on renal handling of vancomicina in rats. *J Pharm. Sci. 87*, 1173-1176.

Lacroix, D., Sonnier, M., Moncion, A., Cheron, G., Cresteil, T. 1997. Expression of CYP3A in the human liver--evidence that the shift between CYP3A7 and CYP3A4 occurs immediately after birth. *Eur. J Biochem. 247*, 625-634.

Landers, S., Berry, P. L., Kearns, G. L., Kaplan, S. L., Rudolph, A. J. 1984. Gentamicin disposition and effect on development of renal function in the very low birth weight infant. *Dev. Pharmacol Ther. 7*, 285-302.

Lapare, S., Tardif, R., Brodeur, J. 1993. Effect of various exposure scenarios on the biological monitoring of organic solvents in alveolar air. I. Toluene and m-xylene. *Int. Arch. Occup. Environ. Health. 64*, 569-580.

Lapare, S., Tardif, R., Brodeur, J. 1995. Effect of various exposure scenarios on the biological monitoring of organic solvents in alveolar air. II. 1,1,1-Trichloroethane and trichloroethylene. *Int. Arch. Occup. Environ. Health. 67*, 375-394.

Lipscomb, J. C., Poet, T. S. 2008. In vitro measurments of metabolism for application in pharmacokinetic modeling. *Pharmacol. Ther. 118*, 82-103.

Lipscomb, J. C., Teuschler, L. K., Swartout, J., Swan, G. E., Snawder, J. E. 2003. Variance of microsomal protein and cytochrome P450 2E1 and 3A forms in adult human liver. *Toxicol. Mech. Meth. 13*, 45-51.

Malviya, S., Lerman, J. 1990. The blood/gas solubilities of sevoflurane, isoflurane, halothane and serum constituent concentrations in neonates and adults. *Anesthesiology 72*, 793-796.

Meek, M. E., Renwick, A., Ohanian, E., Dourson, M., Lake, B., Naumann, B. D., Vu, V. 2002. Guidelines for application of chemical-specific adjustment factors in dose/concentration-response assessment. *Toxicology 181-182*, 115-120.

Meistelman, C., Saint-Maurice, C., Lepaul, M., Levron, J.C., Loose, J.P., Mac, G. K. 1987. A comparison of alfentanil pharmacokinetics in children and adults. *Anesthesiology. 66*, 13-16.

Naumann, B. D., Silverman, K. C., Dixit, R., Faria, E. C., Sargent, E.V. 2001. Case studies for categorical data-derived adjustment factors. *Hum Ecol Risk Assess. 7*, 61-105.

Nelson, W.E. 1991. Textbook of Pediatrics, W.B. Sauders Company, New York NY.

Nong, A., Krishnan, K. 2007. Estimation of interindividual pharmacokinetic variability factor for inhaled volatile organic chemicals using a probability-bounds approach. *Regul. Toxicol. Pharmacol. 48*, 93-101.

Nong, A., McCarver, D. G., Hines, R. N., Krishnan, K., 2006. Modeling interchild differences in pharmacokinetics on the basis of subject-specific data on physiology and hepatic CYP2E1 levels: a case study with toluene. *Toxicol. Appl. Pharmacol. 214*, 78-87.

Pelekis, M., Gephart, L. A., Lerman, S. E. 2001. Physiological-model-based derivation of the adult and child pharmacokinetic intraspecies uncertainty factors for volatile organic compounds. *Regul. Toxicol. Pharmacol. 33*, 12-20.

Pelekis, M., Krewski, D., Krishnan, K. 1997. Physiologically based algebraic expressions for predicting steady-state toxicokinetics of inhaled vapors. *Toxicol. Meth. 7*, 205-225.

Pelekis, M., Krishnan, K. 2004. Magnitude and mechanistic determinants of the interspecies toxicokinetic uncertainty factor for organic chemicals. *Regul. Toxicol. Pharmacol. 40*, 264-271.

Pelekis, M., Nicolich, M. J., Gauthier, J. S. 2003. Probabilistic framework for the estimation of the adult and child toxicokinetic intraspecies uncertainty factors. *Risk Anal. 23*, 1239-1255.

Poulin, P., Krishnan, K. 1996. A mechanistic algorithm for predicting blood:air partition coefficients of organic chemicals with the consideration of reversible binding in hemoglobin. *Toxicol Appl. Pharmacol. 136*, 131-137.

Price, K., Haddad, S., Krishnan, K. 2003a. Physiological modeling of age-specific changes in the pharmacokinetics of organic chemicals in children. *J Toxicol. Environ Health A. 66*, 417-433.

Price, P. S., Conolly, R. B., Chaisson, C. F., Gross, E. A., Young, J. S., Mathis, E. T., Tedder, D. R., 2003b. Modeling interindividual variation in physiological factors used in PBPK models of humans. *Crit Rev Toxicol. 33*, 469-503.

Reinhardt, D., Berdel, D., Heimann, G., Kusenbach, G., Von, B. A., Johnson, E., Steinijans, V. W., Staudinger, H. 1987. Steady state pharmacokinetics, metabolism and

pharmacodynamics of theophylline in children after unequal twice-daily dosing of a new sustained-release formulation. *Chronobiol. Int. 4*, 369-380.

Ronis, M. J. J., Lindros, K. O., Ingelman-Sundberg, M., 1996. The CYP2E family, IN: C Ioannides (Ed.), *Cytochrome P450 Metabolic and Toxicological Aspects*. CRC Press, Boca Raton FL, 211-240.

Rosen, J. P., Danish, M., Ragni, M. C., Saccar, C. L., Yaffe, S. J., Lecks, H. I. 1979. Theophylline pharmacokinetics in the young infant. *Pediatrics. 64*, 248-251.

Saarenmaa, E., Neuvonen, P. J., Fellman, V., 2000. Gestational age and birth weight effects on plasma clearance of fentanyl in newborn infants. *J Pediatr. 136*, 767-770.

Santeiro, M. L., Christie, J., Stromquist, C., Torres, B. A., Markowsky, S.J. 1997. Pharmacokinetics of continuous infusion fentanyl in newborns. *J Perinatol. 17*, 135-139.

Sarangapani, R., Gentry, P. R., Covington, T. R., Teeguarden, J. G., Clewell, H. J., III. 2003. Evaluation of the potential impact of age- and gender-specific lung morphology and ventilation rate on the dosimetry of vapors. *Inhal. Toxicol 15*, 987-1016.

Shimada, T., Yamazaki, H., Mimura, M., Inui, Y., Guengerich, F. P. 1994. Interindividual variations in human liver cytochrome P-450 enzymes involved in the oxidation of drugs, carcinogens and toxic chemicals: studies with liver microsomes of 30 Japanese and 30 Caucasians. *J Pharmacol Exp. Ther. 270*, 414-423.

Simons, F. E., Simons, K. J. 1978. Pharmacokinetics of theophylline in infancy. *J Clin. Pharmacol. 18*, 472-476.

Sonnier, M., Cresteil, T. 1998. Delayed ontogenesis of CYP1A2 in the human liver. *Eur. J Biochem. 251*, 893-898.

Statistics Canada. 2003. Canadian community health survey, cycle 2.1. http://www.statcan.gc.ca/cgi-bin/imdb/p2SV.pl?Function=getSurvey&SurvId=

3226&SurvVer=0&SDDS=3226&InstaId=15282&InstaVer=2&lang=en&db=imdb&adm=8&dis=2. Last accessed: February 1st 2010.

Thomas, R. S., Bigelow, P. L., Keefe, T. J., Yang, R. S. 1996. Variability in biological exposure indices using physiologically based pharmacokinetic modeling and Monte Carlo simulation. *Am Ind. Hyg. Assoc. J. 57*, 23-32.

U.S. EPA. 2002. A review of the reference dose and reference concentration process. Risk Assessment Forum. EPA/630/P-02/00F. Washington DC.

U.S. EPA. 2004. Toxicological Review of Boron and Compounds. Integrated Risk Information System (IRIS). EPA 634/04/052. Washington DC.

Veulemans, H., Masschelein, R. 1978. Experimental human exposure to toluene. II. Toluene in venous blood during and after exposure. *Int Arch Occup. Environ Health. 42*, 105-117.

Valcke, M., Krishnan, K. 2009. Physiologically Based Pharmacokinetic Modeling in the Risk assessment of Developmental Toxicants, IN: D. K. Hansen, B. D. Abbott (Eds.), *Developmental Toxicology*. Informa Health Care, New York NY, pp. 243-274.

Valcke, M., Krishnan, K. 2011. Evaluation of the impact of the exposure route on the human kinetic adjustment factor. *Regul. Toxicol. Pharmacol. 59*, 258-269.

Vincent, J., Teng, R., Dogolo, L. C., Willavize, S.A., Friedman, H. L. 1997. Effect of trovafloxacin, a new fluoroquinolone antibiotic, on the steady-state pharmacokinetics of theophylline in healthy volunteers. *J Antimicrob. Chemother. 39 (SupplB)*, 81-86.

Wang, Y., Moss, J., Thisted, R. 1992. Predictors of body surface area. *J Clin Anesth. 4*, 4-10.

Winter, M. E, 2010. Basic clinical pharmacokinetics, Fifth ed. Lippincott Williams and Wilkins, Baltimore MD, p. 35.

FIGURE CAPTIONS

Figure 4.1 : Illustration of the development of the HKAF matrix, for systemic exposure to CYP1A2 substrates, on the basis of C_{blood}. The results of the Monte Carlo simulation for each subpopulation (here when E = 0.3 and Pb = 300, left panel) are used to determine the highest HKAF value for this combination of E and Pb. Applying this procedure to each Pb value generates one column of the matrix, *i.e.* corresponding to E = 0.3 (right). Repeating the procedure for each E value yields the complete matrix.

Figure 4.2 : Validation of the steady-state algorithm on the basis of predicted *vs* experimental steady-state blood concentrations (dots) of CYP2E1 substrates (VOCs), (A), CYP1A2 substrate (theophylline) and CYP3A4 substrates (fentanyl and alfentanil), (B), and antibiotics mainly cleared (>73 %) by renal filtration, (C). Data on the volunteer (body weight and height, when available) were entered along with exposure conditions as input data, and parent compound's blood concentration was computed. Upper diagonal line represent predicted = 2 × experimental value; middle diagonal line represent predicted = experimental value; lower diagonal line represent predicted = 0.5 × experimental value; error bars indicate the range of experimental data, where available: (A) Laparé *et al.* (1993, 1995), Veulemans and Masschelein (1978); B) Bachmann *et al.* (1990, 1993), Davy *et al.* (1999), Giacoia *et al.* (1976), Gonzalez *et al.* (1994), Gotz *et al.* (1994), Jonkman *et al.* (1991), Meistelman *et al.* (1987), Reinhardt *et al.* (1987), Saarenmaa *et al.* (2000), Santeiro *et al.* (1997), Simons and Simons (1978), Vincent *et al.* (1997); C) Asbury *et al.* (1993), Borin *et al.* (1990), Healy *et al.* (1987), Kildoo *et al.* (1989), Landers *et al.* (1984)).

Figure 4.3 : C_{blood}-based HKAF matrices as a function of hepatic extraction ratios in a 70 kg adult (E) and blood:air partition coefficient (Pb), for the systemic exposure to

substrates metabolised by the CYP2E1 (A), CYP1A2 (B), CYP3A4 (C) and ADH (D) enzymatic pathways. The subpopulation in which the highest HKAF was observed, and subsequently reported in the matrix, is indicated by a contrast code applied to the indicated value: Adults (black type on white background); Elderly (white type on black background); Neonates (black type on grey background).

Figure 4.4 : Average adult *vs* average neonate ratios of body weight-adjusted systemic clearance, for CYP1A2 metabolic pathway, for each combination of E and Pb. Areas of exceedance of the 3.16 default factor, for systemic exposure, is indicated with dotted lines.

Figure 4.5 : C_{blood}-based HKAF matrices as a function of hepatic extraction ratio in a 70 kg adult (E) and blood:air partition coefficient (Pb), for the inhalation exposure to substrates metabolised by the CYP2E1 (A), CYP1A2 (B), CYP3A4 (C) and ADH (D) pathways. The subpopulation in which the highest HKAF was observed, and subsequently captured in the matrix, is indicated by a contrast code applied to the indicated value: Neonates (black type on grey background); Pregnant women (white type on grey background).

Figure 4.6 : Sensitivity analysis of C_{blood} to the physiological parameters in the various subpopulations for six clearance-based scenarios: A) pulmonary clearance >>> hepatic clearance; B) pulmonary clearance ≈ perfusion-limited hepatic clearance; C) pulmonary clearance ≈ enzyme-limited hepatic clearance; D) pulmonary clearance <<< perfusion-limited hepatic clearance; E) pulmonary clearance <<< enzyme-limited hepatic clearance; F) renal clearance dominating. The sensitivity indices were calculated as the change in C_{blood} for a 10 % change in the value of input parameter

(body height, BH; body surface area, BSA; body weight, BW; enzyme content, CYP, glomerular filtration rate, GFR; blood flows to kidneys, Qk; blood flow to liver, Ql; alveolar ventilation rate, Qp; liver volume, Vl).

Figure 4.7 : Sensitivity analysis of C_{blood} to the enzyme content (or activity for ADH) in adults (A) and neonates (B) for different blood:air (Pb) values and hepatic extraction ratios. Hepatic extraction ratios in neonates were those obtained when setting the hepatic extraction ratio in adults as indicated, except for CYP1A2, where values in neonates (0.07, 0.12, 0.23 and 0.39) were obtained by setting the values in adults to 0.5, 0.7, 0.8 and 0.95, respectively.

Figure 4.8 : Contribution, expressed as percentage, of the renal clearance to the body weight-adjusted systemic clearance in adults for hypothetical chemicals exhibiting various E and Pb values.

Figure 4.1

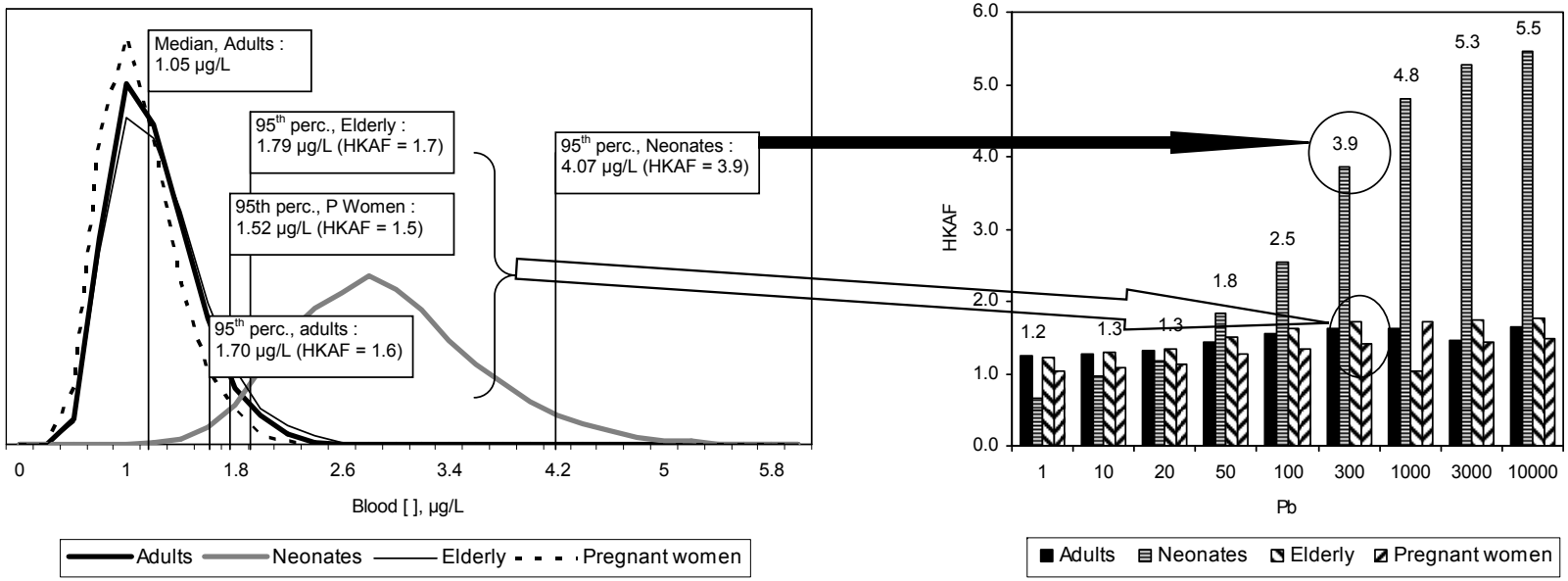
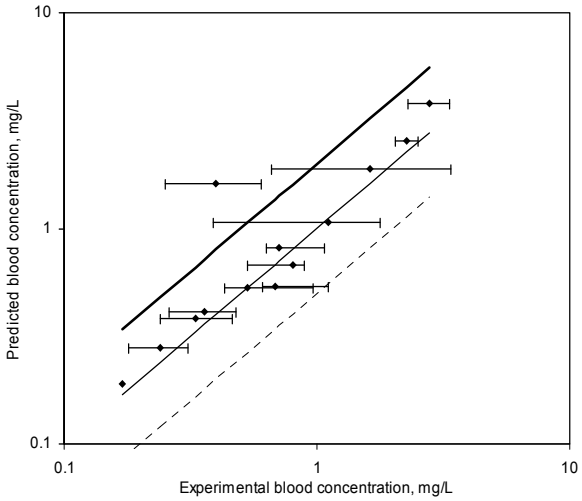
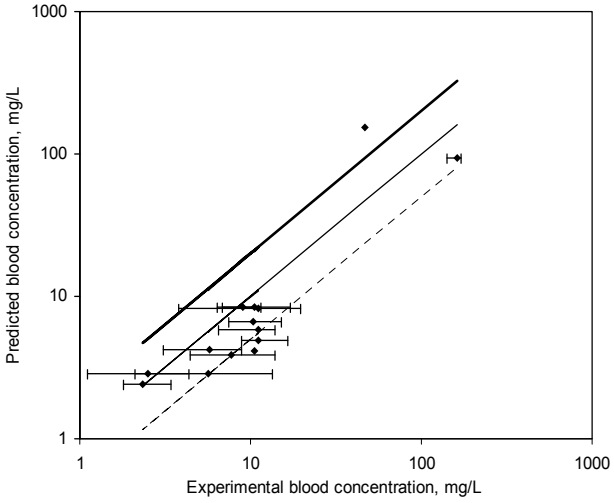

Median, Adults :
1.05 µg/L
95th perc., Elderly :
1.79 µg/L (HKAF = 1.7)
95th perc., P Women :
1.52 µg/L (HKAF = 1.5)
95th perc., adults :
1.70 µg/L (HKAF = 1.6)
95th perc., Neonates :
4.07 µg/L (HKAF = 3.9)
0
1
1.8
2.6
3.4
4.2
5
5.8
Blood [ ], µg/L
Adults
Neonates
Elderly
Pregnant women
HKAF
6.0
5.0
4.0
3.0
2.0
1.0
0.0
1.2
1.3
1.3
1.8
2.5
3.9
4.8
5.3
5.5
1
10
20
50
100
300
1000
3000
10000
Pb
Adults
Neonates
Elderly
Pregnant women

Figure 4.2

a)



b)



c)

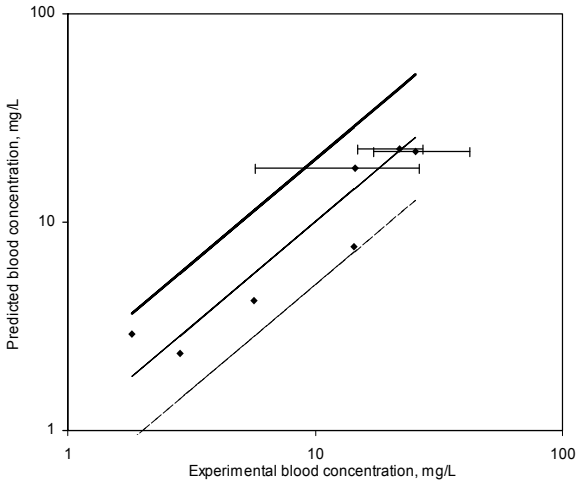


Figure 4.3

a) CYP2E1

E / Pb	0.01	0.1	0.2	0.3	0.5	0.7	0.8	0.9	0.99
1	1.3	1.3	1.2	1.2	1.2	1.2	1.2	1.2	1.2
10	1.2	1.2	1.3	1.3	1.3	1.3	1.3	1.3	1.2
20	1.3	1.3	1.3	1.3	1.4	1.3	1.3	1.3	1.3
50	1.3	1.4	1.4	1.5	1.6	1.5	1.4	1.4	1.4
100	1.3	1.4	1.6	1.8	1.9	1.6	1.4	1.4	1.4
300	1.4	1.8	2.1	2.3	2.3	1.8	1.5	1.4	1.4
1000	1.8	2.1	2.4	2.5	2.4	1.9	1.5	1.4	1.4
3000	2.0	2.3	2.5	2.7	2.4	1.9	1.5	1.4	1.4
10,000	2.0	2.4	2.5	2.7	2.4	1.9	1.5	1.4	1.4

<--------Never greater than 3.16---------->

b) CYP1A2

E / Pb	0.01	0.1	0.2	0.3	0.5	0.7	0.8	0.9	0.99
1	1.2	1.2	1.3	1.2	1.2	1.2	1.2	1.2	1.2
10	1.2	1.3	1.3	1.3	1.3	1.3	1.2	1.3	1.2
20	1.2	1.3	1.3	1.3	1.5	1.7	1.7	1.5	1.3
50	1.3	1.3	1.5	1.8	2.3	2.6	2.5	2.1	1.4
100	1.3	1.6	2.1	2.5	3.2	3.5	3.2	2.4	1.4
300	1.5	2.4	3.2	3.9	4.6	4.5	3.8	2.8	1.4
1000	2.0	3.1	4.1	4.8	5.5	5.1	4.4	2.9	1.4
3000	2.2	3.5	4.5	5.3	6.0	5.4	4.3	3.0	1.4
10,000	2.3	3.6	4.7	5.5	6.1	5.4	4.5	3.0	1.4

<----Greater than 3.16----->

c) CYP3A4

E / Pb	0.01	0.1	0.2	0.3	0.5	0.7	0.8	0.9	0.99
1	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2
10	1.2	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.2
20	1.2	1.3	1.3	1.3	1.4	1.3	1.3	1.3	1.3
50	1.3	1.3	1.4	1.5	1.5	1.4	1.4	1.4	1.4
100	1.3	1.4	1.5	1.6	1.7	1.5	1.4	1.4	1.4
300	1.4	1.7	1.7	2.0	1.9	1.6	1.5	1.4	1.4
1000	1.8	2.0	2.2	2.2	2.0	1.6	1.5	1.4	1.4
3000	2.0	2.1	2.3	2.3	2.1	1.7	1.5	1.4	1.4
10,000	2.1	2.2	2.3	2.3	2.1	1.6	1.5	1.4	1.4

<---------Never greater than 3.16---------->

d) ADH

E / Pb	0.01	0.1	0.2	0.3	0.5	0.7	0.8	0.9	0.99
1	1.3	1.2	1.2	1.3	1.2	1.2	1.2	1.2	1.2
10	1.2	1.2	1.2	1.3	1.2	1.2	1.3	1.3	1.3
20	1.2	1.2	1.3	1.3	1.3	1.3	1.3	1.3	1.3
50	1.3	1.3	1.3	1.4	1.6	1.5	1.4	1.4	1.4
100	1.3	1.3	1.6	1.8	1.9	1.6	1.4	1.4	1.4
300	1.4	1.8	2.1	2.3	2.2	1.8	1.5	1.4	1.4
1000	1.9	2.2	2.5	2.6	2.4	1.9	1.5	1.4	1.4
3000	2.0	2.3	2.6	2.6	2.5	1.9	1.6	1.4	1.4
10,000	2.1	2.4	2.6	2.7	2.5	2.0	1.6	1.4	1.4

<---------Never greater than 3.16---------->

Figure 4.4

CYP1A2

E Pb	0.0	0.1	0.2	0.3	0.5	0.7	0.8	0.9	1.0
1	0.5	0.5	0.5	0.5	0.6	0.6	0.6	0.6	0.5
10	0.6	0.6	0.7	0.8	1.0	1.1	1.1	1.0	0.6
20	0.6	0.8	0.9	1.1	1.3	1.4	1.4	1.2	0.7
50	0.7	1.0	1.3	1.6	2.0	2.1	2.0	1.5	0.7
100	0.8	1.3	1.7	2.1	2.7	2.6	2.3	1.7	0.7
300	1.0	1.7	2.4	2.9	3.5	3.2	2.7	1.8	0.7
1000	1.2	2.1	2.9	3.5	4.0	3.5	2.9	1.9	0.7
3000	1.2	2.2	3.0	3.7	4.2	3.6	2.9	1.9	0.7
10,000	1.2	2.2	3.1	3.7	4.2	3.6	2.9	1.9	0.7

Figure 4.5

a) CYP2E1

E / Pb	0.01	0.1	0.2	0.3	0.5	0.7	0.8	0.9	0.99
1	1.0	1.0	1.0	1.1	1.1	1.1	1.1	1.1	1.1
10	1.1	1.3	1.4	1.5	1.7	1.8	1.7	1.6	1.4
20	1.3	1.5	1.7	1.9	2.2	2.3	2.1	1.8	1.5
50	1.6	2.0	2.4	2.7	3.1	2.9	2.6	2.0	1.6
100	2.0	2.6	3.1	3.5	3.8	3.3	2.8	2.2	1.7
300	2.8	3.6	4.2	4.6	4.4	3.7	3.0	2.2	1.7
1000	3.6	4.3	4.8	5.1	4.7	3.9	3.0	2.2	1.7
3000	4.0	4.5	5.1	5.3	4.9	3.9	3.1	2.3	1.7
10,000	4.1	4.8	5.2	5.4	5.2	3.9	3.1	2.3	1.7

<----Greater than 3.16---->

b) CYP1A2

E_{ad} / Pb	0.01	0.1	0.2	0.3	0.5	0.7	0.8	0.9	0.99
1	1.0	1.0	1.0	1.1	1.1	1.1	1.1	1.1	1.1
10	1.1	1.3	1.4	1.6	1.9	2.1	2.2	2.2	1.5
20	1.3	1.5	1.8	2.1	2.6	3.0	3.1	2.8	1.7
50	1.6	2.2	2.8	3.3	4.3	4.9	4.8	4.1	1.9
100	2.0	3.0	3.9	4.8	6.2	6.6	6.2	4.9	2.0
300	2.9	4.7	6.3	7.6	9.0	8.9	8.0	5.7	2.1
1000	3.9	6.4	8.2	9.9	11.2	10.6	8.8	6.1	2.1
3000	4.4	7.0	9.2	10.7	12.0	11.0	9.0	6.1	2.0
10,000	4.5	7.4	9.7	11.2	12.3	11.0	9.0	6.1	2.1

<--------Greater than 3.16---------->

c) CYP3A4

E / Pb	0.01	0.1	0.2	0.3	0.5	0.7	0.8	0.9	0.99
1	1.0	1.0	1.0	1.1	1.1	1.1	1.1	1.1	1.1
10	1.1	1.4	1.4	1.5	1.6	1.7	1.6	1.6	1.4
20	1.3	1.4	1.7	1.8	2.1	2.1	1.9	1.7	1.5
50	1.6	2.0	2.3	2.6	2.8	2.6	2.3	1.9	1.6
100	2.0	2.5	2.9	3.2	3.3	2.9	2.5	2.0	1.7
300	2.8	3.4	3.9	4.0	3.9	3.2	2.7	2.1	1.7
1000	3.7	4.1	4.4	4.6	4.2	3.3	2.8	2.1	1.7
3000	4.0	4.4	4.6	4.6	4.1	3.3	2.8	2.2	1.7
10,000	4.2	4.5	4.6	4.7	4.3	3.4	2.7	2.2	1.7

<----Greater than 3.16----->

d) ADH

E / Pb	0.01	0.1	0.2	0.3	0.5	0.7	0.8	0.9	0.99
1	1.0	1.0	1.0	1.1	1.1	1.1	1.0	1.1	1.1
10	1.3	1.3	1.4	1.5	1.7	1.8	1.1	1.6	1.4
20	1.5	1.5	1.7	1.9	2.2	2.2	1.3	1.8	1.5
50	2.0	2.0	2.4	2.8	3.1	2.9	1.6	2.1	1.7
100	2.6	2.6	3.2	3.6	3.7	3.3	2.0	2.2	1.7
300	3.6	3.6	4.2	4.6	4.5	3.7	2.9	2.3	1.8
1000	4.4	4.4	4.9	5.2	4.9	3.8	3.7	2.4	1.8
3000	4.7	4.7	5.3	5.3	5.0	4.0	4.1	2.4	1.8
10,000	4.9	4.9	5.3	5.4	5.1	4.0	4.4	2.4	1.8

<---------Greater than 3.16------>

Figure 4.6

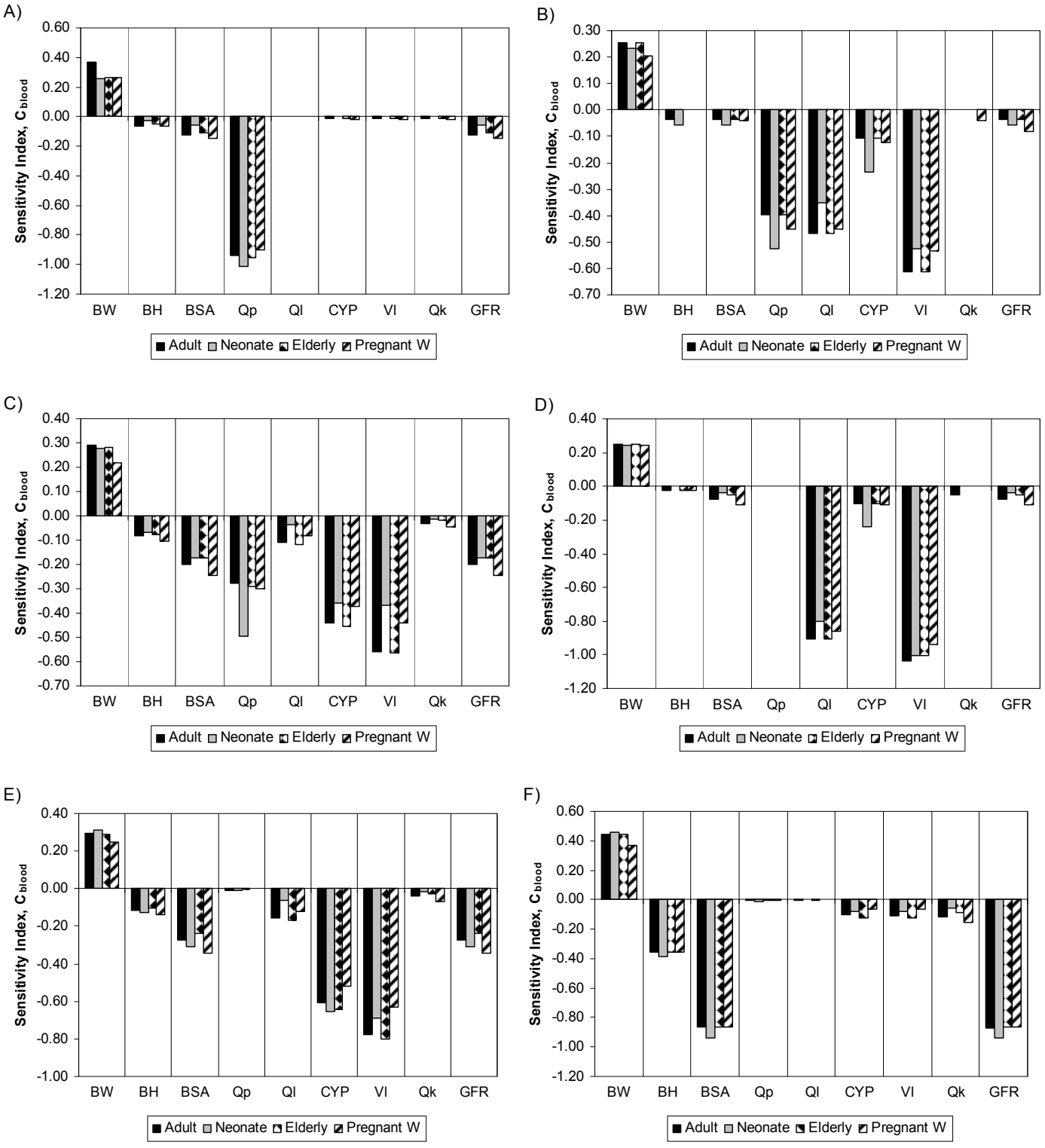
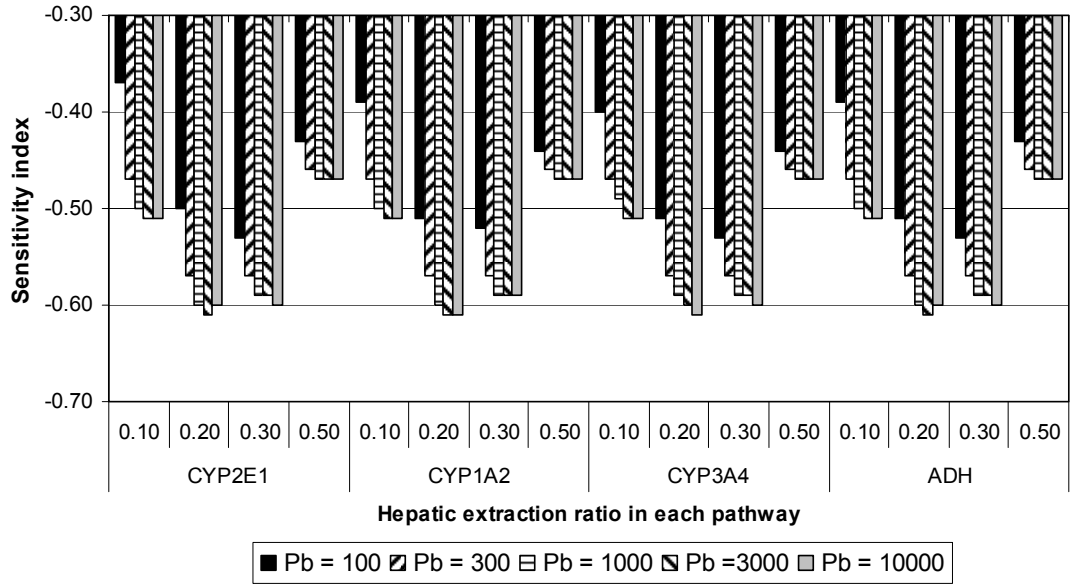

A)
B)
C)
D)
E)
F)
Sensitivity Index, C_{blood}
BW
BH
BSA
Qp
Ql
CYP
Vl
Qk
GFR
Adult
Neonate
Elderly
Pregnant W

Figure 4.7

a)


-0.30
-0.40
-0.50
-0.60
-0.70
Sensitivity index
0.10 0.20 0.30 0.50 0.10 0.20 0.30 0.50 0.10 0.20 0.30 0.50 0.10 0.20 0.30 0.50
CYP2E1
CYP1A2
CYP3A4
ADH
Hepatic extraction ratio in each pathway
Pb = 100 Pb = 300 Pb = 1000 Pb =3000 Pb = 10000

a)

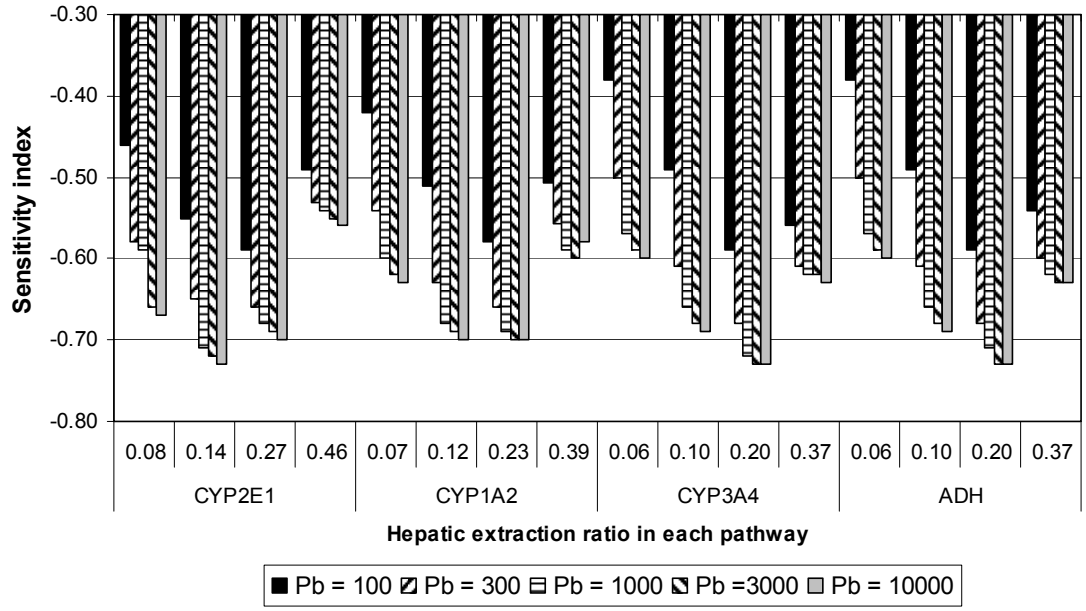

-0.30
-0.40
-0.50
-0.60
-0.70
-0.80
Sensitivity index
0.08 0.14 0.27 0.46 0.07 0.12 0.23 0.39 0.06 0.10 0.20 0.37 0.06 0.10 0.20 0.37
CYP2E1
CYP1A2
CYP3A4
ADH
Hepatic extraction ratio in each pathway
Pb = 100 Pb = 300 Pb = 1000 Pb =3000 Pb = 10000

Figure 4.8

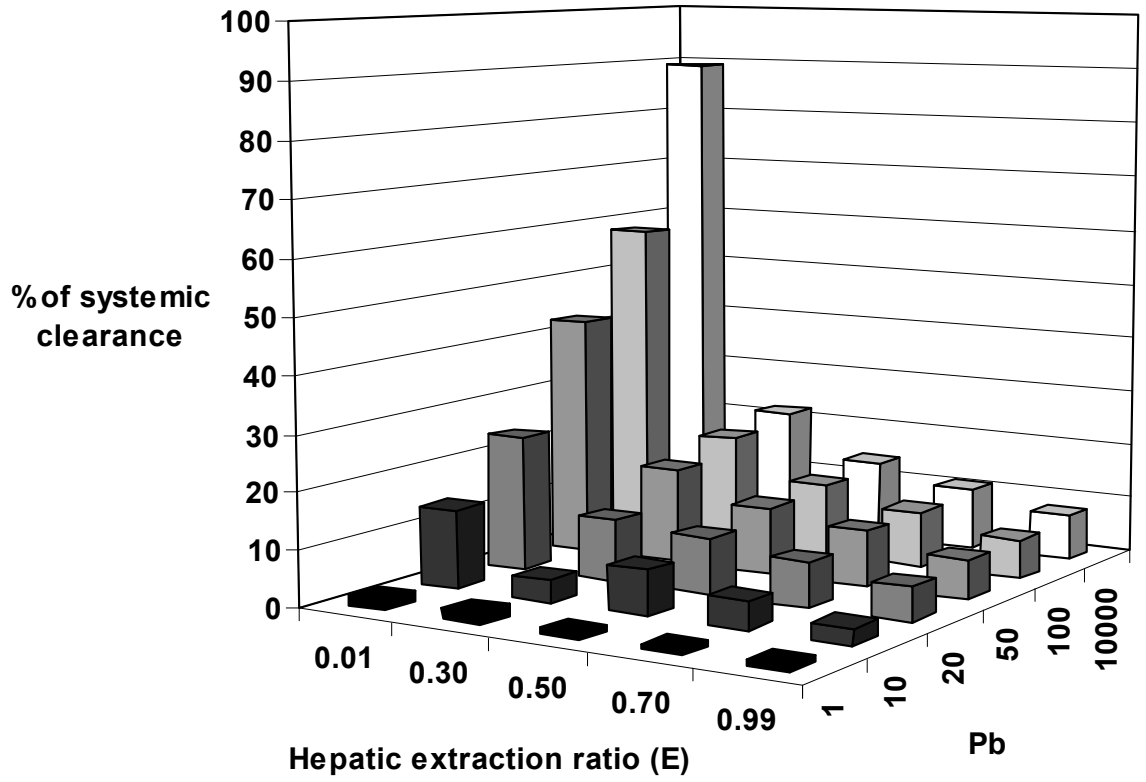

100
90
80
70
60
50
40
30
20
10
0
% of systemic clearance
0.01
0.30
0.50
0.70
0.99
Hepatic extraction ratio (E)
1
10
20
50
100
10000
Pb

Table 4-I : Distributions for the input parameters used in the steady-state algorithm to compute HKAFs

Parameter \ Subpopulation	Adults (18–64 yr)	Neonates (0–30 d)	Elderly (65–90 yr)	Pregnant women (29 yr, 20th week)	References
Distributions[a]					
Body Weight (kg; mean ± SD, range)[b]	76 ± 17, 37–152	4 ± 1, 2–7	72 ± 16, 33–155	70 ± 18, 36–152	P^3M, Johnsrud *et al.*, 2003;
gain in pregnancy (kg; mean± SD)[c]				5 ± 4	ICRP, 2002
Body Height (cm; mean ± SD, range)[b]	167 ± 10, 144–198	46 ± 16, 35–80	164 ± 10, 138–190	161 ± 7, 132–182	P^3M, Nelson's Textbook of Pediatrics
Enzymes content (mean ± SD, range)					
CYP2E1 content (pmol/mg MSP)[d]	49 ± 2, 11–130	18 ± 1, 1–56	49 ± 2, 11–130	49 ± 2, 11–130	Johnsrud *et al.*, 2003; Lipscomb *et al.*, 2003
CYP1A2 content (pmol/mg MSP)	42 ± 23, 7–111	1.43 ± 0.53, 0.37–2.49	42 ± 23, 7–111	42 ± 23, 7–111	Sonnier and Cresteil, 1998; Shimada *et al.*, 1994
CYP3A4 content (pmol/mg MSP)	96 ± 51, 18–247	24 ± 10, 3–45	96 ± 51, 18–247	96 ± 51, 18–247	Lacroix *et al.*, 1997; Shimada *et al.*, 1994
ADH (fraction of adult's activity)[e]	1 ± 0.25, 0.5–1.5	0.25 ± 0.14, 0.03–0.53	1 ± 0.25, 0.5–1.5	1 ± 0.25, 0.5–1.5	Sarangapani *et al.*, 2003
GFR (ml/min-1.73 m^2; mean ± SD, range)	117 ± 9, 87–154	44 ± 20,–84[f]	93 ± 7, 69–121	182 ± 14, 135–240	DeWoskin and Thompson, 2008; Faustman and Ribeiro, 1990
Coefficients of variations of "variability terms" for given body weight and body height[g]					
Qp, Ql, Qk	13 % for all				
Vl	19 % for all				

a): All distributions are lognormal, unless specified otherwise. b): Body weight and body height were correlated as a function of population distribution of body mass index in Canada (r = 0.6, BMI = 14–42, Statistics Canada, 2003) in order to avoid unrealistic combinations during the Monte Carlo Simulations. c): Normal distribution. d) Geometric mean and GSD, MSP = microsomal protein. e) : Fraction of activity in adults. Mean based on Sarangapani *et al.* (2003), SD determined assuming the same coefficient of variation as the mean of CYP2E1, 1A2 and 3A4 coefficients of variation. f): Truncated superiorly at mean + 2 SD. g): Normal distributions centered on the value of 1, truncated at ± 2 SD, based on Valcke and Krishnan (2011), and Thomas *et al.* (1996).

Table 4-II : Determination of pathway-specific intrinsic clearance in adults, adjusted as a function of liver volume, and enzyme hepatic content for CYPs.

E in adults	Required intrinsic clearance[a)]	Pathway-specific adjusted intrinsic clearance in average adult ($Clint_{ad}$)			
		CYP2E1[b)]	CYP1A2[b)]	CYP3A4[b)]	ADH[c)]
0.01	0.8	0.01	0.01	0.01	0.6
0.1	8.5	0.1	0.2	0.06	6.1
0.2	19.0	0.3	0.3	0.14	13.8
0.3	32.6	0.5	0.6	0.25	23.6
0.5	76.1	1.1	1.3	0.57	55.1
0.7	177.5	2.6	3.1	1.34	128.6
0.8	304.2	4.5	5.3	2.30	220.4
0.9	684.5	10.1	11.8	5.17	496.0
0.99	7529.0	111.6	129.9	56.83	5455.8

a): Intrinsic clearance, in L/h. In order to obtain desired E values in adults, a liver blood flow of 76 L/h was used, on the basis of the equations of Price *et al.* (2003b) applied to a 70 kg adult.

b): In L/h-pmol.L/mg MSP, obtained by dividing the Clint (a) by the product of adult mean content of CYP (Table 4-I) and liver volume, the latter being determined on the basis of the equations of Price *et al.* (2003b) applied to a 70 kg adult (1.38 L)

c): In L/h-L of liver, obtained by dividing the Clint by the adult liver volume, as determined on the basis of the equations of Price *et al.* (2003b) applied to a 70 kg adult (1.38L)

Table 4-III: Synthesis of the HKAF obtained for each pathway, exposure route and dose metrics, in each subpopulation investigated

HKAF basis \ E range	Systemic exposure				Inhalation exposure							
	CYP 1A2		All other pathways[a)]		CYP 2E1		CYP 1A2		CYP 3A4		ADH	
	0.01–0.5[b)]	0.5–0.99[b)]	0.01–0.5[b)]	0.5–0.99[b)]	0.01–0.5[b)]	0.5–0.99[b)]	0.01–0.5[b)]	0.5–0.99[b)]	0.01–0.5[b)]	0.5–0.99[b)]	0.01–0.5[b)]	0.5–0.99[b)]
C_{blood}												
Neonates												
Pb =1–100	0.6–3.2	0.7–3.5	0.6–1.9	0.6–1.9	1.0–3.8	1.0–3.8	1.0–6.2	1.1–6.6	1.0–3.3	1.1–3.3	1.0–3.7	1.0–3.7
Pb = 100–10,000	1.1–6.1	1.0–6.1	1.0–2.7	0.8–2.5	2.0–5.4	1.6–5.2	2.0–12.3	2.0–12.3	2.0–4.7	1.6–4.3	2.6–5.4	1.6–5.1
Other subpopulations												
Pb =1–100	1.0–1.6	1.0–1.6	0.8–1.6	1.0–1.6	1–1.7	1.0–1.7	1–1.8	1.0–1.8	1–1.7	1.0–1.7	1.0–1.6	1–1.7
Pb = 100–10,000	1.0–1.8	1.3–1.7	1.0–1.8	1.2–1.7	1.2–1.9	1.4–1.9	1.2–1.9	1.4–1.9	1.2 - 1.9	1.4–1.9	1.3–1.9	1.2–1.8
RAM												
Neonates												
Pb =1–100	0.03–0.3	0.7–0.1	0.2–0.6	0.3–0.7	0.8–1.3	0.9–1.5	0.1–0.5	0.1–1.4	0.5–1.2	0.6–1.5	0.5–1.2	0.5–1.5
Pb = 100–10,000	0.1–0.4	0.3–0.7	0.4–0.9	0.6–0.7	1.2–2.0	1.3–1.5	0.1–0.8	0.5–1.5	0.7–1.4	1.2–1.5	0.8–1.4	0.7–1.6
Other subpopulations												
Pb =1–100	1.2–2.3	1.2–1.5	1.1–2.2	1.1–1.5	1.3–2.1	1.8–1.0	1.3–2.4	1.0–1.8	1.3–2.3	1.0–1.8	1.2–1.6	1.2–1.8
Pb = 100–10,000	1.3–2.3	1.3–1.4	1.1–2.3	1.3–1.4	1.4–2.3	1.0–1.8	1.4–2.4	1.0–1.8	1.4–2.4	1.0–1.8	1.3–1.7	1.3–1.9

a) CYP2E1, CYP3A4 and ADH. b) E range

Abbreviations : ADH, alcohol dehydrogenase; C_{blood}: Steady-state arterial blood concentration; CYP, cytochrome P-450; E, hepatic extraction ratio in average adult of 70 kg; HKAF, human kinetic adjustment factor; Pb, blood :air partition coefficient; RAM, rate of metabolism.

Appendix A : Average systemic dose metrics for CYP2E1 and CYP1A2 pathways in each subpopulation, for nine combinations of hepatic extraction ratio (E) and Pb, covering the range of the cases evaluated in this study

	Adult			**Neonate**			**Elderly**			**Pregnant Women**		
E	0.01	0.5	0.99	0.01	0.5	0.99	0.01	0.5	0.99	0.01	0.5	0.99
Dose metrics												
Pathway												
C_{blood} (µg/L)												
CYP2E1												
Pb = 1	0.1	0.1	0.1	0.04	0.04	0.03	0.1	0.1	0.1	0.1	0.1	0.1
Pb = 100	2.1	0.6	0.4	2.1	0.6	0.2	3.0	0.6	0.4	1.9	0.6	0.3
Pb = 10,000	4.1	0.7	0.4	4.6	0.7	0.2	4.9	0.7	0.4	2.9	0.6	0.3
CYP1A2												
Pb = 1	0.1	0.1	0.06	0.04	0.04	0.03	0.1	0.1	0.06	0.1	0.1	0.1
Pb = 100	2.7	0.6	0.4	2.2	1.6	0.2	3	0.6	0.4	1.9	0.6	0.3
Pb = 10,000	4.1	0.7	0.4	5.0	2.8	0.3	4.9	0.7	0.4	2.9	0.6	0.3
RAM (µg/h-L of liver)												
CYP2E1												
Pb = 1	0.04	1.8	3.4	0.01	0.5	1.7	0.04	1.8	3.3	0.03	1.5	3.0
Pb = 100	1.4	16.9	18.9	0.4	8.3	10.1	1.6	17.1	19.0	1.0	16.3	19.1
Pb = 10,000	2.2	18.4	19.8	0.9	9.7	10.7	2.6	18.7	19.9	1.5	18.1	20.2
CYP1A2												
Pb = 1	0.02	1.8	3.4	< 0.01	0.1	1.4	0.04	1.8	3.3	0.03	1.5	3.0
Pb = 100	1.4	16.9	18.9	0.04	2.9	9.9	1.6	17.1	19.0	1.1	16.3	19.1
Pb = 10,000	2.2	18.4	19.8	0.1	5.0	10.6	2.7	18.7	19.9	1.6	18.0	20.2

Appendix B : Average inhalation dose metrics for CYP2E1 and CYP1A2 pathways in each subpopulation, for nine combinations of hepatic extraction ratio (E) and Pb, covering the range of cases evaluated in this study

	Adult			Neonate			Elderly			Pregnant women		
E	0.01	0.5	0.99	0.01	0.5	0.99	0.01	0.5	0.99	0.01	0.5	0.99
Dose metrics and pathway												
C_{blood} (µg/L)												
CYP2E1												
Pb = 1	10	9	8	10	10	8	10	9	8	10	9	9
Pb = 100	359	83	47	555	149	51	403	85	47	330	97	55
Pb = 10,000	557	90	49	1233	174	53	669	92	50	490	107	58
CYP1A2												
Pb = 1	10	9	8	10	10	9	10	9	8	10	9	9
Pb = 100	359	83	47	575	426	62	402	85	47	330	97	55
Pb = 10,000	556	90	49	1332	738	66	668	92	50	490	107	58
RAM (µg/h-L of liver)												
CYP2E1												
Pb = 1	5	248	454	2	140	452	5	249	455	5	263	506
Pb = 100	191	2276	2547	110	2207	2713	214	2335	2585	176	2784	3254
Pb = 10,000	297	2479	2671	243	2588	2857	356	2549	2711	261	3079	3441
CYP1A2												
Pb = 1	5	248	454	0.2	18	370	5	249	455	5	263	506
Pb = 100	194	2276	2547	11	774	2653	217	2335	2585	178	2784	3254
Pb = 10,000	301	2479	2671	25	1340	2827	361	2549	2712	265	3079	3441

5 Article IV: *Assessing the impact of child/adult differences in oral bioavailability on the human kinetic adjustment factor for ingested toxicants*

Valcke, M. et Krishnan, K.

ASSESSING THE IMPACT OF CHILD/ADULT DIFFERENCES IN ORAL BIOAVAILABILITY ON THE HUMAN KINETIC ADJUSTMENT FACTOR FOR INGESTED TOXICANTS

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Abstract

The objective of this study was to evaluate the impact of interindividual differences in oral bioavailability on the magnitude of the human kinetic adjustment factor (HKAF). This factor is intended to replace the default value of 3.2 in non-cancer risk assessment and aims to account for interindividual variability in toxicokinetics. Steady-state equations that compute arterial blood concentrations (CAss) and liver volume-adjusted rates of metabolism (RAMs) for oral exposure to chemicals, accounting for the hepatic first-pass effect, were used. The results predicted by these equations for an average adult were validated by full-blown physiologically-based toxicokinetic models. They were then solved with Monte Carlo simulations to generate distributions of CAss and RAM values in neonates, infants, toddlers, and adults for theoretical CYP2E1 and CYP1A2 substrates exhibiting a range of blood:air partition coefficients (Pb: 1–10,000) and hepatic extraction ratios (E: 0.01–0.99) in an average adult. For each combination of E and Pb, HKAF values were computed as the ratio of the 95th percentile of dose metrics for each subpopulation to the 50th percentile value in adults and reported in an enzyme-specific matrix. HKAFs > 3.2 were observed only based on CAss for various Pb values, in neonates when $E \geq 0.3$ (CYP2E1 substrates; max.: 6.3) and in both neonates and infants when $E \geq 0.1$ and 0.7, respectively (CYP1A2 substrates; max.: 28.3). In all cases, including considering RAM, the HKAF was lower than 3.2. Overall, this study characterized the impact of child/adult differences in oral bioavailability on the variability of CAss and resulting HKAF.

Keywords: Bioavailability, CYP2E1/1A2, Human kinetic adjustment factor, Interindividual variability, Monte-Carlo simulations, Oral exposure, Risk assessment, Steady-state toxicokinetics.

LIST OF ABBREVIATIONS AND ACRONYMS

ADH	**alcohol dehydrogenase**
CAss	**arterial blood concentration at steady-state**
CSAF	**chemical-specific adjustment factor**
CYP	**cytochrome P-450**
E	**hepatic extraction ratio in average adult**
E_{ren}	**renal extraction ratio**
GSD	**geometric standard deviation**
GST	**glutathione-s-transferase**
HKAF	**human kinetic adjustment factor**
ING	**ingested dose**
IPCS	**International Programme on Chemical Safety**
IVF	**interindividual variability factor**
Km	**Michaelis-Menten constant**
MSP	**microsomal protein**
Pb	**blood:air partition coefficient**
PBTK	**physiologically-based toxicokinetic**
Qc	**cardiac output**
Qk	**kidney blood flow**
Ql	**liver blood flow**
Qp	**alveolar ventilation rate**
RAM	**rate of metabolism**
RfC	**reference concentration**
RfD	**reference dose**
SD	**standard deviation**
Vmax	**maximum rate of metabolism**
Vl	**volume of liver**

5.1 Introduction

For non-cancer risk assessment, steady-state toxicokinetic analyses have been used to evaluate the impact of interindividual variability on internal dose metrics (Ginsberg *et al.*, 2005; Nong and Krishnan, 2007; Pelekis *et al.*, 2001; Valcke and Krishnan, 2011b). This allows evaluating the magnitude and adequacy of the toxicokinetic component of the 10-fold interindividual variability factor (IVF) that is used to derive chronic reference doses (RfD) or concentrations (RfC) (Dourson *et al.*, 1996; U.S. EPA, 2002). Given that a default value of 3.2 (*i.e.* $\sqrt{10}$) has been attributed to this component based on pharmaceutical data (Dorne and Renwick 2005; Renwick and Lazarus, 1998), evaluation or replacement of the default value can be made by quantifying chemical-specific adjustment factors (CSAFs) described by the International Programme on Chemical Safety (IPCS, 2005). Using this method, the CSAF for interindividual variability in toxicokinetics, also referred to as the human kinetic adjustment factor (HKAF), can be determined based on experimental or modeled upper and median percentile data from population and subpopulation distributions of pharmacokinetic parameters or internal dose metrics (IPCS, 2005; Meek *et al.*, 2002). In this regard, steady-state equations appear particularly useful as they have been shown to significantly simplify the estimation of internal dose metrics as compared to complete physiologically-based toxicokinetic (PBTK) models when simulating continuous exposures to xenobiotics (Andersen, 1981; Aylward *et al.*, 2010; Bogen, 1988; Bogen and Gold, 1997; Bogen and Hall, 1989; Bogen and McKone, 1988; Chiu and White, 2006; Csanady and Filser, 2001; Pelekis *et al.*, 1997, 2001).

For inhalation exposures, steady-state solutions have been shown to generate almost identical results as PBTK models (Pelekis *et al.*, 1997, 2001). For oral exposures, however, this comparison has not yet been performed extensively. The impact of hepatic "first pass effect", or pre-systemic clearance, on the oral bioavailability of chemicals has not been systematically quantified by steady-state equations derived from PBTK models. Given that the presystemic clearance is particularly important only for oral exposures (Gibaldi and Perrier, 1982), accounting for it when determining the HKAF for RfD derivation appears

desirable. Indeed, the interindividual variability of this phenomenon has not been systematically quantified to-date for environmental contaminants. Although some experimental studies with drugs suggest that this variability is not very important (e.g. Edwards and Stoeckel, 1992; Fanta *et al.*, 2007; Hassan *et al.*, 1994), Beck *et al.* (2002) have considered a six-fold greater oral bioavailability of lead in 2-yr old children as compared to pregnant women in a modeling exercise. Besides, CYP2E1, CYP1A1/1A2 and other hepatic enzymes involved in the biotransformation of environmental contaminants (Ronis, 1996) are less developed in young children when compared to adults, particularly during the first year of life (Johnsrud *et al.*, 2003; Sonnier and Cresteil, 1998). This enzyme deficiency affects the metabolic capacity of children (e.g., Ginsberg *et al.*, 2005; Nong *et al.*, 2006; Valcke and Krishnan, 2011a) and likely differentially impacts the steady-state blood concentration of ingested chemicals in this subpopulation as compared to adults, and thus the resulting HKAF. Recently, Valcke and Krishnan (2011b) computed the HKAF for inhalation and BW-adjusted systemic exposure to chemicals exhibiting various physico- and biochemical properties, but not for ingestion exposures. The objectives of the current study were: 1) validate steady-state equations that account for the first pass effect and oral bioavailability of ingested chemicals; and 2) use these equations to evaluate the magnitude of the HKAF for chronic oral exposures based on differences in the distributions of internal dose metrics between children and adults.

5.2 Methods

The methodology involved 1) the validation of steady-state equations for ingested chemicals with full-blown PBTK models published in the literature; 2) solving these equations with Monte Carlo simulations to generate distributions of internal dose metrics in children and adults for chemicals exhibiting various physico- and biochemical characteristics; and 3) computation of the HKAF following the IPCS (2005) approach.

5.2.1 Steady-state equations for ingested chemicals

The bioavailable fraction of a dose of xenobiotics absorbed through the gut after its ingestion corresponds to the fraction of that dose that escapes the pre-systemic "first pass" effect in the liver before entering the systemic circulation, and equals (1-E), where E is the hepatic extraction ratio. Thus, the systemic ingested dose is expressed as "dose × (1-E)" (Gibaldi and Perrier, 1982; Gillette, 1980; Rowland and Tozer, 1995). The mathematical derivation of this expression from PBTK models is described in the Appendix. It is used as the numerator of the steady-state equation applied to compute arterial blood concentration (CAss) as described previously for systemic exposure (Valcke and Krishnan 2011b). Precisely, CAss is calculated as the systemic dose rate divided by the systemic clearance, the latter being composed of the hepatic (Ql×E), pulmonary (Qp/Pb) and renal ($Qk \times E_{ren}$) clearance:

$$CAss = \frac{DR \times (1 - E)}{(Ql \times E) + (Qp/Pb) + (Qk \times E_{ren})} \quad (1)$$

… where DR is the ingested dose rate, Qk, Ql, Qp and E_{ren} are respectively the renal blood flow, liver blood flow, alveolar ventilation rate and the renal extraction ratio. The calculation of the liver volume (Vl)-adjusted rate of metabolism (RAM) (Valcke and Krishnan, 2011b) was expanded to account for the first-pass effect, as follows:

$$RAM = \frac{(CAss \times Ql \times E) + (DR \times E)}{Vl} \quad (2)$$

Input parameters, with exception of E and E_{ren} were calculated for a given individual from the body weight and height, using equations modified from Price *et al.* (2003) and described previously (Valcke and Krishnan, 2011b). E and E_{ren} were determined based on the intrinsic clearance and the glomerular filtration rate, respectively (Valcke and Krishnan, 2011b).

5.2.2 Validation of the steady-state equations

For an average 70 kg adult exposed continuously to 1 µg/kg-d, the results given by Eqs. 1 and 2 were compared to those given at t = 500 h by PBTK models for chloroform, bromoform, tri- and tetrachloroethylene (Valcke and Krishnan, 2011a), toluene, m-xylene (Tardif *et al.*, 1995), carbon tetrachloride (Delic *et al.*, 2000), vinyl chloride (Reitz *et al.*, 1996), styrene (Ramsey and Andersen, 1984), benzene (Haddad *et al.*, 2001), and methyl chloroform (Lu *et al.*, 2008).

5.2.3 Simulations of distribution of internal dose metrics in children and adults and computation of the HKAF for ingestion exposure

To compute the CAss and RAM distributions for a continuous ingestion exposure of 1 µg/kg-d, Monte Carlo simulations involving 5000 iterations were applied to Eqs. 1 and 2 in adults, as well as in three subgroups of children: neonates (0–30 d), infants (1–12 mo), and toddlers (1–3 yr). In the process, an absorption fraction through the gut of 100% was assumed for every subpopulation, and the physiological data as presented in Table 5-I were used. Calculations were performed for theoretical CYP2E1 and CYP1A2 (as a surrogate for CYP1A1) substrates exhibiting nine different values of Pb (range: 1–10,000) and nine different values of E in an average adult (range: 0.01–0.99). Thus, the intrinsic clearance for a given theoretical chemical was set as a function of the targeted E value by accounting for an average adult's liver blood flow, i.e. 76 L/hr for a 70 kg adult, as per the equations of Price *et al*. (2003). Then, the intrinsic clearance for a given individual in any subpopulation ($Clint_{ind}$) was calculated based on liver volume- and enzyme content-adjusted intrinsic clearance in an average adult ($Clint_{ad}$), The latter was obtained by dividing the intrinsic clearance determined above for each E value by the average adult's liver volume (1,38 L, as per the equations of Price *et al*. (2003)) and enzyme content (Table 5-I). Therefore, based on the premise that intrinsic clearance in any individual relates proportionally to the intrinsic clearance in average adult as a function of the ratio of the total amount of relevant

hepatic enzymes $Clint_{ind}$ was computed on the basis of $Clint_{ad}$ and the individual's liver volume and enzyme content, as per Eq. 3. This entire approach is detailed in Valcke and Krishnan (2011b).

$$Clint_{subpop} = Clint_{ad} \times Vl_{subpop} \times [enzyme]_{subpop} \quad (3)$$

From the distributions of internal dose metrics generated for substrates exhibiting each one of the 81 possible combinations of E and Pb values, the HKAF was computed as the ratio of the 95th percentile value of each subpopulation to the 50th percentile value in adults (IPCS, 2005). The greatest HKAF obtained considering each subpopulation was reported in a "Pb *vs* E", 9 × 9 HKAF matrix, as described previously (Valcke and Krishnan, 2011b).

5.3 Results

5.3.1 Validation of steady-state equations for ingestion exposure

Fig. 5.1 shows that the CAss (a) and RAM (b) values computed from the steady-state equations for a 70 kg adult are in good agreement with the values predicted by PBTK models at t = 500 h. The computed CAss slightly exceed the PBTK predictions, an expected result given that, particularly for highly lipophilic chemicals such as carbon tetrachloride and tetrachloroethylene, steady-state may not be achieved at t = 500 h. However, the computed RAM values are almost identical to the PBTK-based predictions.

5.3.2 Subpopulation-specific oral bioavailability of CYP2E1 and CYP1A2 substrates

In Table 5-II, the impact of the hepatic enzyme content on the intrinsic clearance (see Eq. 3) and resulting E and bioavailability in each subpopulation is shown. For poorly extracted

substrates (*i.e.* E ≤ 0.3 in average adult) of the rapidly developing CYP2E1 enzymatic pathway, the neonate E values are significantly lower (*i.e.* at least two-fold) than for the adult, likely because the reduced enzyme levels limit metabolism. However, the bioavailable fractions remain comparable. For greater hepatic extraction ratios, the child/adult difference in bioavailability increases in favor of the neonate, as a two- to three-fold difference compared to adults is observed when E in average adult is at least equal to 0.8. For substrates of the slowly developing CYP1A2 enzymatic pathway, the adult-to-neonate difference in E and oral bioavailability increases as compared o CYP2E1 pathway. Indeed, E is always at least three-fold lower in the average neonate when compared to the adult, except for nearly completely extracted chemicals (E = 0.99 in average adult). Thus, when E ≥ 0.5 in average adult, bioavailability in neonates is at least two-fold greater, but up to 23-fold greater when this E value reaches 0.99. E values and bioavailability in infants and toddlers were comparable to those in the average adult for CYP2E1 substrates, whereas this was the case for CYP1A2 substrates in toddlers only.

5.3.3 Internal doses and HKAF matrices for CYP2E1 and CYP1A2 following an ingestion exposure

Differences in oral bioavailability between children and adults can lead to corresponding differences in internal dose metrics. Assuming an oral exposure of 1 µg/kg-d, the mean CAss and RAM values obtained in each subpopulation are shown in Table 5-III. These values account for a range of three E values in an average adult (0.01, 0.5 and 0.99) and three Pb values (1, 100 and 10,000). Although adults exhibit greater CAss values regardless of the enzyme considered for E = 0.01 and Pb = 10,000, neonates exhibit greater CAss values in most cases. Greatest CAss values for neonates were also obtained for CYP1A2 substrates only when Pb = 1. In every case, infants exhibited CAss values between those of neonates and adults. Results in toddlers, however, were comparable to adults. The RAM values for adults were significantly greater in all cases analyzed.

Fig. 5.2 shows the CAss-based HKAF matrices for ingestion exposure to CYP2E1 and CYP1A2 substrates. HKAF increases steadily with increasing Pb and E values. The greatest HKAF is obtained in neonates at Pb = 300 for E = 0.01, and then at a lower Pb value for every increase in the E value, until E = 0.7. Otherwise, adults exhibited the greatest HKAFs. When Pb = 1000, the 3.2 default value could be exceeded at a lower E value for CYP1A2 (0.1) than for CYP2E1 (0.3). The results also suggest that, when E is increased, the default value can be exceeded at lower Pb values. Thus, HKAFs as high as 6.3 (CYP2E1) and 28.3 (CYP1A2) were projected for neonates. When $E \geq 0.7$ and $Pb \geq 100$, the default value could be exceeded for CYP1A2 substrates in infants (max.: = 4.4). In toddlers, however, the default value might never be exceeded; the highest values obtained were 1.6 and 2.6 for CYP2E1 and CYP1A2 substrates, respectively (not shown). On the basis of RAM, HKAF was always greater in adults and varied slightly, from 1.4 to 2.2, depending on the type of CYP, E and Pb values considered (not shown).

5.4 Discussion

This study successfully used steady-state equations for ingestion exposures to generate distributions of relevant internal dose metrics in children and adults and to compute HKAF for hypothetical CYP2E1 and CYP1A2 substrates. The results obtained point out that, under certain circumstances, the default value of 3.2 can be exceeded on the basis of parent compound's dose metrics. This finding was observed for both types of substrates in neonates but also for CYP1A2 substrates in infants and included chemicals that exhibit hepatic extraction ratios as low as 0.1 in average adults (for CYP1A2) (Fig. 5.2b). Importantly, the first-pass effect is mediated by hepatic enzymes that are only partially developed in these subpopulations, resulting in significantly smaller pre-systemic metabolism and much greater bioavailability of ingested doses (Table 5-II). Consequently, the actual "systemic dose" is greater, impacting both the CAss and resulting HKAF values. The delayed development of CYP1A2 means that infants are still deficient in this enzyme when compared to adults. This deficit is much less important in toddlers and is not pronounced for the rapidly developing CYP2E1 (Table 5-I). Thus, for CYP1A2 substrates

only, the oral bioavailability, CAss and corresponding HKAF values also remain significantly greater in infants (Table 5-II).

From a toxicokinetic standpoint, adults may be more sensitive than children to compounds with low extraction ratios and Pb < 100 (Fig. 5.2). Low extraction ratios result in small differences in the systemic bioavailability of ingested chemicals between adults and children (Table 5-II); however, relatively low Pb values signify a more efficient pulmonary clearance in children because of their greater BW-adjusted pulmonary ventilation, resulting in lower blood concentrations and HKAF values (Valcke and Krishnan, 2011a, 2011b). Adults are always more sensitive when using RAM values (Table 5-III). This is because of their fully developed hepatic enzymes allowing efficient biotransformation of parent compounds into their metabolites contrary to children for whom these enzymes are not fully developed (Valcke and Krishnan, 2011a, 2011b).

The impact of pre-systemic clearance during oral exposure on the HKAF is shown in Fig. 5.3. This figure shows the mean (a) and maximum (b) HKAF values obtained for each targeted E value in the matrices of the current study and previous work where only systemic clearance was taken into account. Differences are minimal for low hepatic extraction ratios which may explain the low variability in oral bioavailability observed in studies with drugs (Edwards and Stoeckel, 1992; Fanta *et al.*, 2007; Hassan *et al.*, 1994). However, the difference increases steadily with increasing E values. This difference remains within a 4-fold factor for CYP2E1 substrates, but increases up to ≈ 10-fold for CYP1A2 substrates due to the slower development of this enzyme in children. Thus, refering solely to variability in systemic clearance may underestimate significantly the true interindividual variability in internal dose metrics for ingested chemicals, and resulting HKAFs. Given that the development of CYP2E1 is similar to other enzymatic pathways, such as CYP3A4 (Lacroix *et al.*, 1997), alcohol dehydrogenase (ADH, Sarangapani *et al.*, 2003) and even possibly glutathione-s-transferase (GST, Strange *et al.*, 1989), child/adult

differences in oral bioavailability and resulting oral HKAFs determined for these enzymatic pathways would likely be comparable.

The results of the present study on adult-child differences are comparable to those on internal dose or HKAF values reported for short-term exposures in the literature for substances with similar E and Pb values. Given the steady-state assumptions followed here, these results are relevant to chronic exposures for which RfDs are derived. Using the simulated 95th percentile value of the 24-h area under the curve (AUC) for blood concentration *vs* time, HKAF values of 4.9, 7.4 and 2.6 were obtained in neonates for the following CYP2E1 substrates, respectively: chloroform (E ≈ 0.9, Pb = 7.4), bromoform (E ≈ 0.9, Pb = 102), and trichloroethylene (E ≈ 0.6, Pb = 9.2) (Valcke and Krishnan, 2011a). Walker *et al.* (2007) obtained an HKAF of 4.9 for acrylamide (E ≈ 0.1, Pb > 10,000), a CYP2E1/GST substrate, based on the simulated 99th percentile value of the 24-h AUC in neonates. Clewell *et al.* (2004) obtained a neonate/adult simulated blood concentration ratio of 1.9 for ADH-metabolized isopropanol (E ≈ 0.9, Pb = 848). Finally, Dorne *et al.* (2001) estimated a 2.9-fold adults/neonate ratio for the mean experimental clearances of CYP1A2-metabolized theophyline (E ≈ 0.1, Pb > 10,000).

Some limitations of this study warrant discussion. First, 100% absorption through the gut was assumed for all subpopulations, although interindividual variability in oral absorption may stem from child/adult differences in the physiology and structure of the intestine. In the latter case, interindividual differences in the expression of intestinal transporters may result in corresponding variability in oral bioavailability (Katsura and Inui, 2003). Age-related differences in the expression of these transporters have been documented in animal tissues (Cheng and Klaassen, 2009; Maher *et al*., 2005; Schiengold *et al*., 2001; St-Pierre *et al*., 2004) but not in the small intestine nor in other human tissues. Some CYP-related metabolism, which thus would be affected by the related ontogeny, has also been demonstrated to act as a barrier to gut absorption, at least for CYP3A drug substrates (Tamura *et al.*, 2003; Thummel *et al.*, 1996). But transporters, additionally of being at least

partly chemical-specific, alter either the influx or the efflux of the xenobiotics through the intestinal wall. Also, steady-state analyses such as those performed here imply that metabolism occurs only in the liver (Pelekis *et al.*, 1997), an assumption that precludes the consideration of intestinal metabolism. As a result, the net impact of the ontogeny of intestinal transporters and CYPs, should sufficient data be available, could hardly be assessed using the approach followed here. Finally, in some studies, (e.g. Haddad *et al.*, 2006, Valcke and Krishnan, 2011a), BW-adjusted oral absorption rates, which thus vary between children and adults, have been used. However, this does not affect steady-state conditions as those considered here, contrary to scenarios involving short-term exposures.

Second, there are other potentially sensitive subpopulations that were not considered. But deficits in hepatic enzymes that occur in children make this population a prime candidate for increased sensitivity with regard to the impact of oral bioavailability. In this regard, other subpopulations that exhibit reduced enzymatic activity due to genetic polymorphisms may be investigated. To our knowledge however, genetic polymorphisms that are significant for population variability in enzymatic activity have not been reported for the CYPs investigated here (Ginsberg *et al.* 2009; Neafsey *et al.*, 2009). This issue would warrant further investigation for other relevant enzymes.

In conclusion, this study has, for the first time, systematically investigated the impact of the differences in oral biovailability of chemicals in children and adults on the HKAF following oral exposure to chemicals. The results obtained suggest that the 3.2-fold default factor that is currently used to account for interindividual variability in toxicokinetics may not be sufficient in all cases to protect neonates during oral exposure to CYP2E1 and CYP1A2 substrates. For the categories of chemicals possessing specific physicochemical and biochemical characteristics then, chemical-specific adjustment factors should be developed to replace defaults in the establishment of toxicological reference values for oral exposure as per the IPCS (2005) approach.

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5.5 References

Andersen, M. E., 1981. A physiologically based toxicokinetic description of the metabolism of inhaled gases and vapors: analysis at steady state. *Toxicol Appl. Pharmacol. 60*, 509-526.

Aylward, L. L., Kirman, C. R., Blount, B. C., Hays, S. M. 2010. Chemical-specific screening criteria for interpretation of biomonitoring data for volatile organic compounds (VOCs)--application of steady-state PBPK model solutions. *Regul. Toxicol. Pharmacol. 58*, 33-44.

Beck, B. D., Mattuck, R. L., Bowers, T. S. 2002. Adult-child differences in the intraspecies uncertainty factor: a case study using lead. *Human Ecol. Risk Assess*. *8*, 877-884.

Bogen, K. T. 1988. Pharmacokinetics for regulatory risk analysis: the case of trichloroethylene. *Regul. Toxicol. Pharmacol. 8*, 447-466.

Bogen, K. T., Gold, L. S. 1997. Trichloroethylene cancer risk: simplified calculation of PBPK-based MCLs for cytotoxic end points. *Regul. Toxicol. Pharmacol. 25*, 26-42.

Bogen, K. T., Hall, L. C. 1989. Pharmacokinetics for regulatory risk analysis: the case of 1,1,1-trichloroethane (methyl chloroform). *Regul. Toxicol. Pharmacol. 10*, 26-50.

Bogen, K. T., McKone, T. E. 1988. Linking indoor air and pharmacokinetic models to assess tetrachloroethylene risk. *Risk Anal. 8*, 509-520.

Cheng, X., Klaassen, C. D. 2009. Tissue distribution, ontogeny, and hormonal regulation of xenobiotic transporters in mouse kidneys. *Drug Metab. Disp. 37*, 2178-2185.

Chiu, W. A., White, P. 2006. Steady-state solutions to PBPK models and their applications to risk assessment I: Route-to-route extrapolation of volatile chemicals. *Risk Anal. 26*, 769-780.

Clewell, H. J., Gentry, P. R., Covington, T. R., Sarangapani, R., Teeguarden, J.G. 2004. Evaluation of the potential impact of age- and gender-specific pharmacokinetic differences on tissue dosimetry. *Toxicol Sci. 79*, 381-393.

Csanady, G. A., Filser, J. G. 2001. The relevance of physical activity for the kinetics of inhaled gaseous substances. *Arch. Toxicol. 74*, 663-672.

Delic, J. I., Lilly, P. D., MacDonald, A. J., Loizou, G. D. 2000. The utility of PBPK in the safety assessment of chloroform and carbon tetrachloride. *Regul. Toxicol. Pharmacol. 32*, 144-155.

DeWoskin, R. S., Thompson, C. M. 2008. Renal clearance parameters for PBPK model analysis of early lifestage differences in the disposition of environmental toxicants. *Regul. Toxicol Pharmacol. 51*, 66-86.

Dorne, J. L., Renwick, A. G. 2005. The refinement of uncertainty/safety factors in risk assessment by the incorporation of data on toxicokinetic variability in humans. *Toxicol. Sci.* 86, 20-26.

Dorne, J. L., Walton, K., Renwick, A. G. 2001. Uncertainty factors for chemical risk assessment: human variability in the pharmacokinetics of CYP1A2 probe substrates. *Food Chem. Toxicol. 39*, 681-696.

Dourson, M. L., Felter, S. P., Robinson, D. 1996. Evolution of science-based uncertainty factors in noncancer risk assessment. *Regul. Toxicol. Pharmacol. 24*, 108-120.

Edwards, D. J., Stoeckel, K. 1992. The pharmacokinetics of new oral cephalosporins in children. *Chemotherapy 38(s2)*, 2–9.

Fanta S., Jönsson, S., Backman, J. T., Karlsson, M. O., Hoppu, K. 2007. Developmental pharmacokinetics of ciclosporin–a population pharmacokinetic study in paediatric renal transplant candidates. *Br. J. Clin. Pharmacol. 64*, 772-784.

Gibaldi, M., Perrier, D. 1982. Pharmacokinetics, Second ed. Marcel Dekker Inc., New York NY.

Gillette, J. R. 1980. Pharmacokinetic factors govering the steady-state concentrations of foreign chemicals and their metabolites. IN: D. Evered, G Lawrenson (Eds.) *Environmental chemicals, enzyme function and human disease*. Ciba Foundation Symposium 76, Glendale CA, pp. 191 - 217.

Ginsberg, G., Smolenski, S., Neafsey, P., Hattis, D., Walker, K., Guyton, K. Z., Johns, D. O., Sonawane, B. 2009. The influence of Genetic Polymorphism on Population Variability in Six Xenobiotic-Metabolizing Enzymes. *J Toxicol. Environ Health B. 12*, 307-333.

Ginsberg, G., Foos, B. P., Firestone, M. P. 2005. Review and analysis of inhalation dosimetry methods for application to children's risk assessment. *J Toxicol. Environ Health A. 68*, 573-615.

Haddad, S., Beliveau, M., Tardif, R., Krishnan, K. 2001. A PBPK modeling-based approach to account for interactions in the health risk assessment of chemical mixtures. *Toxicol. Sci. 63*, 125-131.

Haddad, S., Tardif, G.C., Tardif, R. 2006. Development of physiologically based toxicokinetic models for improving the human indoor exposure assessment to water contaminants: trichloroethylene and trihalomethanes. *J Toxicol. Environ Health A. 69*, 2095-2136.

Hassan, M., Ljungman, P., Bolme, P., Ringden, O., Syruckova, Z., Stary, J., Wallin, I., Kallberg, N. 1994. Busulfan bioavailability. *Blood 84*, 2144-2150.

IPCS. 2005. Chemical-Specific Adjustment Factors (CSAFs) for Interspecies Differences and Human Variability: Guidance Document for the Use of Data in Dose/Concentration-

Response Assessment. WHO/IPCS/01.4, 1-96. International Panel on Chemical Safety, World Health Organisation, Geneva, Switzerland

Johnsrud, E. K., Koukouritaki, S. B., Divakaran, K., Brunengraber, L. L., Hines, R. N., McCarver, D. G. 2003. Human hepatic CYP2E1 expression during development. *J Pharmacol. Exp. Ther. 307*, 402-407.

Katsura, T., Inui, K. 2003. Intestinal absorption of drugs mediated by drug transporters : mechanisms and regulation. *Drug Metab. Pharmacokin. 18*, 1-15.

Lacroix, D., Sonnier, M., Moncion, A., Cheron, G., Cresteil, T. 1997. Expression of CYP3A in the human liver--evidence that the shift between CYP3A7 and CYP3A4 occurs immediately after birth. *Eur. J Biochem. 247*, 625-634.

Lipscomb, J. C., Poet, T. S. 2008. In vitro measurments of metabolism for application in pharmacokinetic modeling. *Pharmacol. Ther. 118*, 82-103.

Lipscomb, J. C., Teuschler, L. K., Swartout, J., Swan, G. E., Snawder, J. E. 2003. Variance of microsomal protein and cytochrome P450 2E1 and 3A forms in adult human liver. *Toxicol. Mech. Meth. 13*, 45-51.

Lu, Y., Rieth, S., Lohitnavy, M., Dennison, J., El-Masri, H., Barton, H. A., Bruckner, J., Yang, R. S. 2008. Application of PBPK modeling in support of the derivation of toxicity reference values for 1,1,1-trichloroethane. *Regul. Toxicol Pharmacol. 50*, 249-260.

Maher, J. M., Slitt, A. L., Cherrington, N. J., Cheng X, Klaassen C. D. 2005. Tissue distribution and hepatic and renal ontogeny of the multidrugresistance-associated protein (Mrp) family in mice. *Drug Metab Dispos. 33*, 947-955.

Meek, M. E., Renwick, A., Ohanian, E., Dourson, M., Lake, B., Naumann, B. D., Vu, V. 2002. Guidelines for application of chemical-specific adjustment factors in dose/concentration-response assessment. *Toxicology. 181-182*, 115-120.

Neafsey, P., Ginsberg, G., Hattis, D., Johns, D. O., Guyton, K. Z., Sonawane, B. 2009. Genetic Polymorphism in CYP2E1: Population Distribution of CYP2E1 Activity. *J Toxicol. Environ Health B. 12*, 362-388.

Nelson, W. E. 1991. Textbook of Pediatrics, W.B. Sauders Company, New York, NY.

Nong, A., Krishnan, K. 2007. Estimation of interindividual pharmacokinetic variability factor for inhaled volatile organic chemicals using a probability-bounds approach. Regul. Toxicol. Pharmacol. *48*, 93-101.

Nong, A., McCarver, D. G., Hines, R. N., Krishnan, K. 2006. Modeling interchild differences in pharmacokinetics on the basis of subject-specific data on physiology and hepatic CYP2E1 levels: a case study with toluene. *Toxicol. Appl. Pharmacol. 214*, 78-87.

Pelekis, M., Gephart, L. A., Lerman, S. E. 2001. Physiological-model-based derivation of the adult and child pharmacokinetic intraspecies uncertainty factors for volatile organic compounds. *Regul. Toxicol. Pharmacol. 33*, 12-20.

Pelekis, M., Krewski, D., Krishnan, K. 1997. Physiologically based algebraic expressions for predicting steady-state toxicokinetics of inhaled vapors. *Toxicol. Meth. 7*, 205-225.

Price, P. S., Conolly, R. B., Chaisson, C. F., Gross, E. A., Young, J. S., Mathis, E. T., Tedder, D. R. 2003. Modeling interindividual variation in physiological factors used in PBPK models of humans. *Crit Rev Toxicol. 33*, 469-503.

Ramsey, J. C., Andersen, M. E. 1984. A physiologically based description of the inhalation pharmacokinetics of styrene in rats and humans. *Toxicol Appl. Pharmacol. 73*, 159-175.

Reitz, R. H., Gargas, M. L., Andersen, M. E., Provan, W. M., Green, T. L. 1996. Predicting cancer risk from vinyl chloride exposure with a physiologically based pharmacokinetic model. *Toxicol Appl. Pharmacol. 137*, 253-267.

Renwick, A. G., Lazarus, N. R. 1998. Human variability and noncancer risk assessment--an analysis of the default uncertainty factor. *Regul. Toxicol. Pharmacol. 27*, 3-20.

Ronis, M. J. J., Lindros, K. O., Ingelman-Sundberg, M. 1996. The CYP2E family. IN: C. Ioannides (Ed.), *Cytochrome P450 Metabolic and Toxicological Aspects*, CRC PRess, Boca Raton FL, pp. 211-240.

Rowland, M., Tozer, T. N. 1995. Clinical pharmacokinetics–concepts and applications, third ed. Lippincott, Williams and Wilkins, Media, PA.

Sarangapani, R., Gentry, P. R., Covington, T. R., Teeguarden, J. G., Clewell, H. J., III. 2003. Evaluation of the potential impact of age- and gender-specific lung morphology and ventilation rate on the dosimetry of vapors. *Inhal. Toxicol 15*, 987-1016.

Schiengold, M., Schwantes, L., Schwartsmann, G., Chies, J. A., Nardi, N. B. 2001. Multidrug resistance gene expression during the murine ontogeny. *Mech Ageing Dev. 122*, 255-270.

Shimada, T., Yamazaki, H., Mimura, M., Inui, Y., Guengerich, F. P. 1994. Interindividual variations in human liver cytochrome P-450 enzymes involved in the oxidation of drugs, carcinogens and toxic chemicals: studies with liver microsomes of 30 Japanese and 30 Caucasians. *J Pharmacol Exp. Ther. 270*, 414-423.

Sonnier, M., Cresteil, T. 1998. Delayed ontogenesis of CYP1A2 in the human liver. *Eur. J Biochem. 251*, 893-898.

St-Pierre, M. V., Stallmach, T., Freimoser Grundschober, A., Dufour, J.F., Serrano, M. A., Marin, J. J, Sugiyama, Y., Meier, P. J. 2004. Temporal expression profiles of organic anion transport proteins in placenta and fetal liver of the rat. *Am J Physiol Regul Integr Comp Physiol. 287*, 1505-16.

Strange, R. C., Howie, A. F., Hume, R., Matharoo, B., Bell, J., Hiley, C., Jones, P., Beckett, G.J. 1989. The development expression of alpha-, mu- and pi-class glutathione S-transferases in human liver. *Biochim. Biophys. Acta. 99*, 186-190

Tamura, S., Tokunaga, Y., Ibuki, R., Amidon, G. L., Sezaki, H., Yamashita, S. 2003. The site-specific transport and metabolism of tacrolimus in rat small intestine. *J Pharmacol Exp. Ther. 306*, 310-316.

Tardif, R., Lapare, S., Charest-Tardif, G., Brodeur, J., Krishnan, K. 1995. Physiologically-based pharmacokinetic modeling of a mixture of toluene and xylene in humans. *Risk Anal. 15*, 335-342.

Thummel, K. E., O'Shea, D., Paine, M. F., Shen, D. S., Kunze, K. L., Perkins, J. D., Wilkinson, G. R. 1996. Oral first-pass elimination of midazolam involves both gastrointestinal and hepatic CYP3A-mediated metabolism. *Clin Pharmacol Ther. 59*, 491-502.

U.S.EPA. 2002. A review of the reference dose and reference concentration process. Risk Assessment Forum. EPA/630/P-02/00F. Washington DC, U.S.A.

Valcke, M., Krishnan, K. 2011a. Evaluation of the impact of the exposure route on the human kinetic adjustment factor. *Regul. Toxicol Pharmacol. 59*, 258-269.

Valcke, M., Krishnan, K. 2011b. Evaluation of the Impact of physico-chemical and biochemical characteristics on the human kinetic adjustment factor for systemic toxicants. *Toxicology 286*, 36-47. doi:10.1016/j.tox.2011.05.003..

Walker, K., Hattis, D., Russ, A., Sonawane, B., Ginsberg, G. 2007. Approaches to acrylamide physiologically based toxicokinetic modeling for exploring child-adult dosimetry differences. *J Toxicol. Environ Health A. 70*, 2033-2055.

FIGURE CAPTIONS

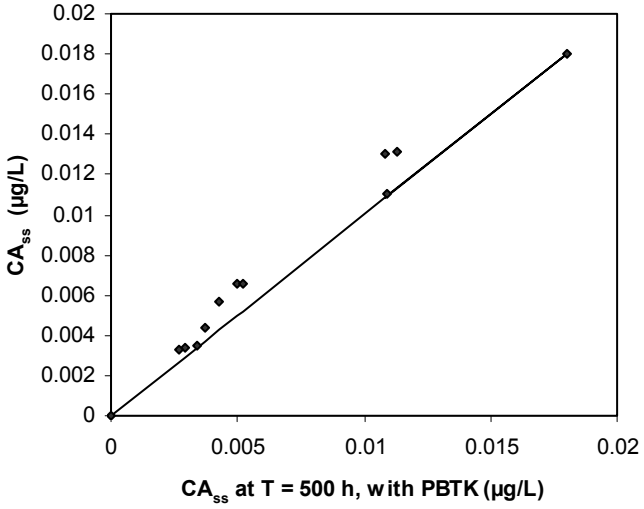
Figure 5.1 : Validation of the steady-state equations for CAss (a) and RAM (b) for ingestion exposure. The values predicted by the equations are compared the values obtained at t = 500 h with PBTK models for 11 chemicals (see Methods) assuming a 70 kg adult undergoing a continuous exposure of 1 µg/kg-d.

Figure 5.2 : CAss-based HKAF matrices as a function of hepatic extraction ratios in a 70 kg adult (E) and blood:air partition coefficient (Pb) following ingestion of CYP2E1 (a) and CYP1A2 (b) substrates. The subpopulations in which the highest HKAFs were observed are indicated in the matrix by a contrast code: Adults (black type on white background); Neonates (black type on grey background). In (b), values in lower case and parenthesis indicate the HKAFs obtained for infants that exceeded the 3.16 default value.

Figure 5.3 : Differences in mean (a) and maximum (b) CAss-based HKAFs by E value. Values calculated in the current study ("ingested") and those calculated for a BW-adjusted systemic dose ("systemic") as detailed in Valcke and Krishnan, 2011b) are shown.

Figure 5.1

a)


0.02
0.018
0.016
0.014
0.012
0.01
0.008
0.006
0.004
0.002
0
CA$_{ss}$ (µg/L)
0
0.005
0.01
0.015
0.02
CA$_{ss}$ at T = 500 h, with PBTK (µg/L)

b)

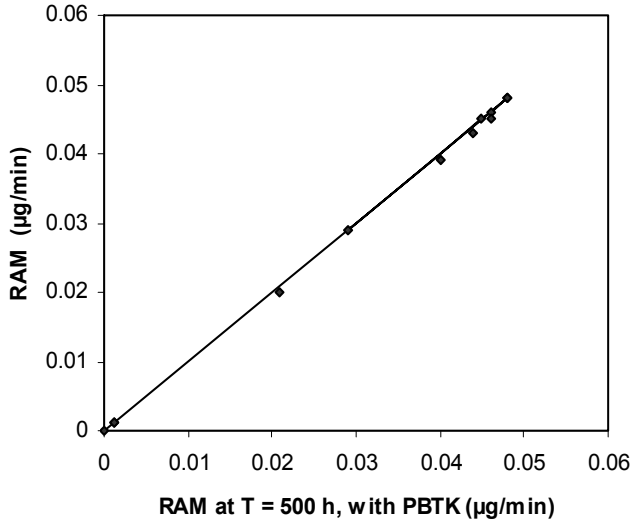

0.06
0.05
0.04
0.03
0.02
0.01
0
RAM (µg/min)
0
0.01
0.02
0.03
0.04
0.05
0.06
RAM at T = 500 h, with PBTK (µg/min)

Figure 5.2

a) CYP2E1

E / Pb	0.01	0.1	0.2	0.3	0.5	0.7	0.8	0.9	0.99
1	1.3	1.3	1.3	1.4	1.5	1.7	2.1	3.2	5.4
10	1.2	1.3	1.4	1.5	1.7	2.6	3.2	4.3	5.7
20	1.2	1.3	1.4	1.6	2.1	3.1	3.8	4.8	5.8
50	1.3	1.3	1.6	1.9	2.9	3.9	4.6	5.1	5.9
100	1.2	1.5	1.9	2.4	3.5	4.4	4.6	5.5	5.8
300	1.4	2.0	2.6	3.1	4.2	5.0	5.3	5.8	6.0
1000	1.8	2.3	2.9	3.5	4.3	5.3	5.2	5.8	6.1
3000	2.0	2.5	3.1	3.6	4.4	5.1	5.4	6.1	5.8
10,000	2.1	2.6	3.1	3.7	4.6	5.2	5.6	5.8	6.3

<----------Greater than 3.16---------->

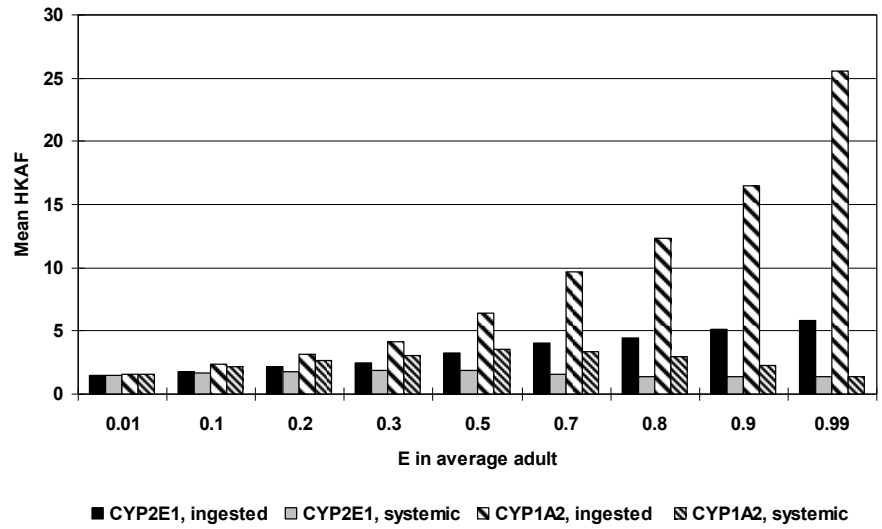
b) CYP1A2

E / Pb	0.01	0.1	0.2	0.3	0.5	0.7	0.8	0.9	0.99
1	1.2	1.3	1.3	1.3	1.5	2.0	2.9	5.1	18.7 (4.2)
10	1.2	1.3	1.4	1.5	2.0	3.5	5.2	8.7 (3.3)	21.8 (4.1)
20	1.2	1.3	1.4	1.7	2.7	4.9	7.1	11.4 (3.6)	24.2 (4.2)
50	1.3	1.4	1.9	2.5	4.3	7.5	10.3 (3.4)	15.4 (3.9)	26.2 (4.3)
100	1.2	1.7	2.5	3.5	6.0	9.8 (3.2)	13.0 (3.6)	18.4 (4.0)	26.5 (4.3)
300	1.5	2.6	3.9	5.2	8.4	13.1 (3.4)	16.3 (3.7)	21.2 (4.1)	27.8 (4.4)
1000	1.9	3.4	5.0	6.6	10.4	14.9 (3.5)	18.4 (3.8)	22.5 (4.2)	28.1 (4.4)
3000	2.2	3.8	5.6	7.1	11.0	15.6 (3.5)	19.3 (3.9)	22.3 (4.1)	28.0 (4.3)
10,000	2.3	4.0	5.8	7.6	11.2	15.5 (3.5)	18.9 (3.8)	22.9 (4.2)	28.3 (4.4)

<----------Greater than 3.16---------->

Figure 5.3

a)


30
25
20
15
10
5
0
Mean HKAF
0.01
0.1
0.2
0.3
0.5
0.7
0.8
0.9
0.99
E in average adult
CYP2E1, ingested
CYP2E1, systemic
CYP1A2, ingested
CYP1A2, systemic

b)

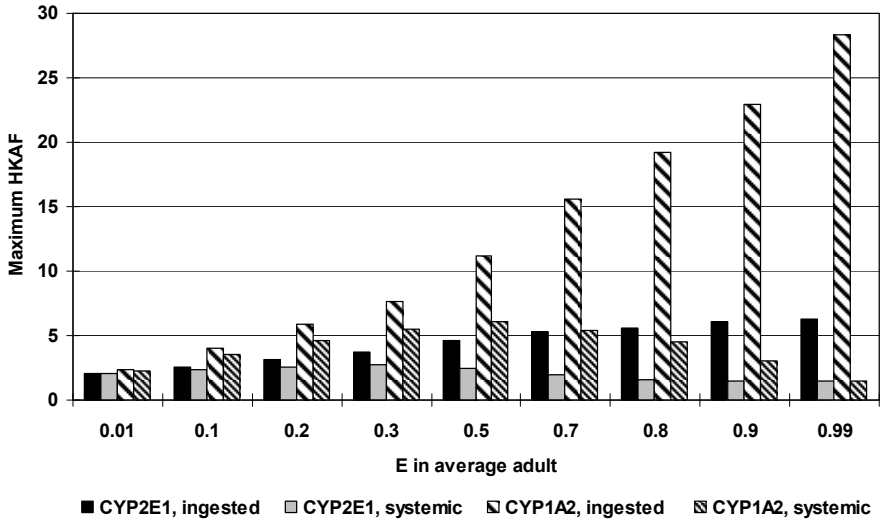

30
25
20
15
10
5
0
Maximum HKAF
0.01
0.1
0.2
0.3
0.5
0.7
0.8
0.9
0.99
E in average adult
CYP2E1, ingested
CYP2E1, systemic
CYP1A2, ingested
CYP1A2, systemic

Table 5-I : Physiological parameters used for the Monte Carlo simulations of internal dose metrics with steady-state equations.

Parameter [a]	Subpopulation	Adults	Neonates	Infants	Toddlers
	Median age (range)	41 (18–64)	14 d (0–30 d)	6.5 mo (1–12 mo)	2 (1–3)
Body weight					
	(Kg, m ± SD, range)[b]	76 ± 17, 37–152	4 ± 1, 2–7	9 ± 2, 3–15	13 ± 2, 7–32
Body height					
	(cm, m ± SD, range)[c]	167 ± 10, 132–198	51 ± 16, 46–80	69 ± 6, 59–88	87 ± 6, 70–106
Hepatic enzyme content					
(pmol/mg MSP, m ± SD, range):					
	CYP2E1[d]	49 ± 2, 11–130	18 ± 14, 1–56	36 ± 21, 10–86	42 ± 18, 18–74
	CYP1A2[e]	42 ± 23, 7–111	1.4 ± 0.5, 0.4–2.5	9.2 ± 2.8, 3.7–14.8	21.8 ± 9.6, 2.6–41
Glomerular filtration rate[f]					
(ml/min-1.73 m^2; mean ± SD, range)		117 ± 9, 87–154	44 ± 20, 4–84	110 ± 18, 74–146	127 ± 9, 109–145

a) Log normal distributions b) From the P^3M Database (Price *et al.*, 2003), except for neonates (Johnsrud *et al.*, 2003). c) From the P^3M Database (Price *et al.*, 2003), except for neonates (Nelson, 1991). d) From Johnsrud *et al.* (2003), except for adults (Geometric mean ± GSD, Lipscomb *et al.*, 2003; Lipscomb and Poet, 2008). f) Mean enzyme content in adults as per Shimada *et al.* (1994). Values in children were calculated on the basis of the ratio of enzyme activity as described by Sonnier and Cresteil (1998). The standard deviations were set to correspond to the same coefficient of variation as in adults. f) Distributions in children estimated from DeWoskin and Thompson (2008), with truncations at ± 2 SD. Distribution in adults based on the age-specific value between 18 and 90 yr, assuming a decrease of 0.67 % per year from 127 ml/min-1.73 m^2. **Abbreviations**: d, days; m, mean; mo, months; MSP, microsomal protein; SD, standard deviation.

Table 5-II : E value and bioavailability of an ingested dose of CYP2E1 and CYP1A2 substrates in an average individual of each subpopulation investigated, for a given E in an average adult.

E in an average adult (Fraction bioavailable)[a)] enzyme	**0.01** (0.99)	**0.1** (0.9)	**0.2** (0.8)	**0.3** (0.7)	**0.5** (0.5)	**0.7** (0.3)	**0.8** (0.2)	**0.9** (0.1)	**0.99** (0.01)
Neonate									
CYP2E1									
E value	0.004	0.04	0.08	0.14	0.27	0.46	0.60	0.77	0.97
Fraction bioavailable[a)]	0.996	0.96	0.92	0.86	0.73	0.54	0.40	0.23	0.03
CYP1A2									
E value	0.0003	0.004	0.01	0.01	0.03	0.07	0.12	0.23	0.77
Fraction bioavailable[a)]	0.9997	0.996	0.99	0.99	0.97	0.93	0.82	0.77	0.23
Infant									
CYP2E1									
E value	0.006	0.07	0.14	0.21	0.39	0.60	0.72	0.85	0.98
Fraction bioavailable[a)]	0.994	0.93	0.96	0.79	0.61	0.40	0.28	0.15	0.02
CYP1A2									
E value	0.002	0.02	0.05	0.09	0.18	0.34	0.47	0.66	0.96
Fraction bioavailable[a)]	0.998	0.98	0.95	0.91	0.82	0.66	0.53	0.34	0.04
Toddler									
CYP2E1									
E value	0.007	0.08	0.16	0.24	0.43	0.64	0.75	0.87	0.99
Fraction bioavailable[a)]	0.993	0.92	0.84	0.76	0.57	0.36	0.25	0.13	0.01
CYP1A2									
E value	0.005	0.05	0.11	0.18	0.34	0.55	0.67	0.82	0.98
Fraction bioavailable[a)]	0.995	0.95	0.89	0.82	0.66	0.45	0.33	0.18	0.02

a) For an ingested dose, computed as 1-E.

Table 5-III : Mean steady-state arterial blood concentrations (CAss) and liver volume-adjusted rates of metabolism (RAM) in children and adults following a continuous ingestion exposure to 1 µg/kg-d of CYP2E1 and CYP1A2 substrates. Nine combinations of hepatic extraction ratios (E) and blood:air partition coefficienst (Pb) were evaluated.

Subpopulation	Adults			Neonates			Infants			Toddlers		
E	0.01	0.5	0.99	0.01	0.5	0.99	0.01	0.5	0.99	0.01	0.5	0.99
Dose metric / enzyme considered												
CAss (µg/L)												
CYP2E1												
Pb = 1	0.1	0.03	0.001	0.04	0.03	0.001	0.04	0.03	0.001	0.1	0.03	0.001
Pb = 100	2.6	0.3	0.004	2.1	0.6	0.009	1.5	0.3	0.004	1.6	0.3	0.003
Pb = 10,000	4.1	0.4	0.004	4.9	0.7	0.009	2.4	0.3	0.004	2.3	0.3	0.003
CYP1A2												
Pb = 1	0.07	0.04	0.001	0.04	0.04	0.008	0.05	0.04	0.002	0.05	0.03	0.001
Pb = 100	2.6	0.4	0.005	2.1	1.6	0.062	1.6	0.6	0.011	1.6	0.4	0.005
Pb = 10,000	4.1	0.4	0.005	5.3	2.8	0.067	2.5	0.8	0.011	2.3	0.5	0.006
RAM (µg/h-L of liver)												
CYP2E1												
Pb = 1	0.3	11.9	22	0.1	3.0	11.0	0.1	6.0	13.5	0.1	7.1	14.7
Pb = 100	1.7	19.3	22.2	0.4	8.3	11.5	0.7	11.1	13.7	0.8	12.5	15.0
Pb = 10,000	2.5	20.2	22.1	0.9	9.7	11.5	1.0	11.8	13.7	1.1	13.1	15.0
CYP1A2												
Pb = 1	0.25	11.2	22	0.004	0.4	8.9	0.03	2.7	13.1	0.09	5.3	14.6
Pb = 100	1.6	19.0	22	0.04	3.0	11.1	0.2	8.2	13.5	0.5	11.2	14.8
Pb = 10,000	2.3	20.0	22.1	0.1	5.0	10.9	0.3	9.5	13.6	0.7	1	14.9

Appendix.

In PBTK models, the steady-state arterial blood concentration (CAss) following chronic exposure to inhaled chemicals is computed from the airborne concentration (Ci) and steady-state venous blood concentration (CVss) as follows (Pelekis *et al.*, 1997):

$$CAss = \frac{Qp \times Ci + Qc \times CVss}{Qc + Qp/Pb} \quad (1)$$

where Qc is the cardiac output, Qp is the alveolar ventilation rate, and Pb is the blood:air partition coefficient. For ingested chemicals, Qp × Ci equals 0 and CVss becomes the sole "source of input" in the above equation. CVss is calculated from the venous blood concentration exiting each tissue (Pelekis *et al.*, 1997). At steady-state, it equals CAss for non-metabolizing tissues, but not for liver, such that (Pelekis *et al.* (1997):

$$CVss = \frac{[CAss \times (Qc - Ql)] + (Ql \times CVlss)}{Qc} \quad (2)$$

Setting Ql = 0.25Qc (Delic *et al.*, 2000), Eq. 2 can be simplified to:

$$CVss = 0.75CAss + 0.25CVlss \quad (3)$$

Given that, at steady-state, the amount of chemical entering and exiting the liver are equal, it can be formulated that the input to the liver (through the arterial blood and the ingested dose ING) equals the sum of the amount metabolized and eliminated by other clearance processes (Figure A-1).

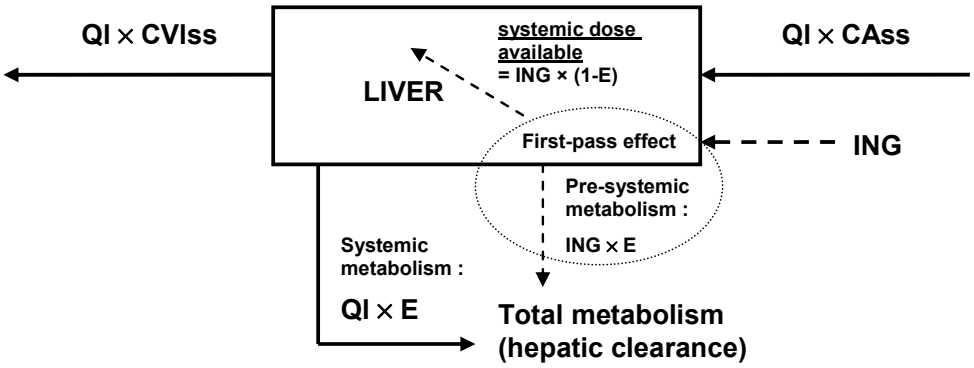


Figure A-1. Illustration of the liver uptake and elimination of chemicals at steady-state following oral dosing. CAss, steady-state arterial blood concentration; CVlss, steady-state liver's venous blood concentration; E, hepatic extraction ratio; ING, ingested dose rate; Ql, blood flow to/from the liver

Accordingly,

$$(CAss \times Ql) + ING = (Ql \times E \times CAss) + (Ql \times CVlss) + (ING \times E) \quad (4)$$

where E is the hepatic extraction ratio. Upon division of all terms of Eq. 4 by Ql, and rearrangement, CVl_{ss} can be isolated as follows:

$$CVlss = CAss + (ING / Ql) - (ING \times E / Ql) - (E \times CAss) \quad (5)$$

Isolating (1-E), the above equation can be rewritten as:

$$CVlss = [CAss + (ING / Ql)] \times (1 - E) \quad (6)$$

Inserting Eq. 6 into Eq. 3, we get:

$$CVss = 0.75CAss + 0.25[(CAss + (ING / Ql)) \times (1 - E)] \quad (7)$$

Thus, Eq. 1 for ingested chemicals becomes:

$$CAss = \frac{Qc \times [0.75CAss + 0.25[(CAss + (ING/Ql)) \times (1 - E)]]}{Qc + Qp/Pb} \quad (8)$$

Rearranging, the above equation yields:

$$Qc \times CAss + CAss \times Qp / Pb = Qc \times 0.75CAss + Qc \times 0.25[(CAss + (ING / Ql)) \times (1 - E)] \quad (9)$$

Simplifying:

$$CAss \times Qp / Pb = Qc \times 0.25ING / Ql – Qc \times 0.25E \times CAss – Qc \times 0.25E \times ING / Ql \quad (10)$$

Given that Ql = 0.25Qc (Delic *et al.*, 2000):

$$CAss \times Qp / Pb + Qc \times 0.25E \times CAss + Qc \times 0.25E \times ING / Ql = ING \quad (11)$$

Simplifying, Eq. 11 becomes:

$$[CAss \times (Qp / Pb + Ql \times E)] + (E \times ING) = ING \quad (12)$$

Rearranging, we obtain:

$$CAss = \frac{ING \times (1 - E)}{(Ql \times E) + (Qp/Pb)} \quad (13)$$

The equation 13, representing the steady-state solution of a PBTK model for ingestion exposure, is similar or identical to that used historically in classical pharmacokinetics (Chiu and White, 2006; Gibaldi and Perrier, 1982; Gillette, 1980; Rowland and Tozer, 1995).

6 Article V: *Modeling the human kinetic adjustment factor for inhalaed volatile organic chemicals: whole population approach vs distinct subpopulation approach*

Valcke, M., Nong, A., et Krishnan, K.

Sous presse, *Journal of Toxicology*

MODELING THE HUMAN KINETIC ADJUSTMENT FACTOR FOR INHALED VOLATILE ORGANIC CHEMICALS: WHOLE POPULATION APPROACH *VS* DISTINCT SUBPOPULATION APPROACH

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Abstract

The objective of this study was to evaluate the impact of whole- and sub-population-related variabilities on the determination of the human kinetic adjustment factor (HKAF) used in risk assessment of inhaled volatile organic chemicals (VOCs). Monte Carlo simulations were applied to a steady-state algorithm to generate population distributions for blood concentrations (CAss) and rates of metabolism (RAMs) for inhalation exposures to benzene (BZ) and 1,4-dioxane (1,4-D). The simulated population consisted of various proportions of adults, elderly, children, neonates and pregnant women as per the demography of the Canadian population. Subgroup-specific input parameters were obtained from the literature and P_3M software. Under the "whole population" approach, the HKAF was computed as the ratio of the entire population's upper percentile value (99^{th}, 95^{th}) of dose metrics to the median value in either the entire population or the adult population. Under the "distinct subpopulation" approach, the upper percentile values in each subpopulation were considered, and the greatest resulting HKAF was retained. CAss-based HKAFs that considered the Canadian demography varied between 1.2 (BZ) and 2.8 (1,4-D) and was marginally affected by the composition of the population. Based on the "distinct subpopulation" approach, the greatest CAss-based HKAF varied between 1.6 (BZ) and 8.5 (1,4-D), and both values were obtained for neonates. In all instances, RAM-based HKAFs did not vary much (1.2-1.6), and pregnant women exhibited the greatest values. Furthermore, the simulation results suggest that depending of the assumptions, the "whole population" -based HKAF covered at the most 73% of the neonates based on CAss, and 86% of pregnant women based on RAM, whereas the "distinct subpopulation"-based HKAF always covered at least 99% of the entire population. Overall, this study evaluated for the first time the impact of underlying assumptions with respect to the interindividual variability considered (whole population or each subpopulation taken separately) when determining the HKAF.

Keywords: Demography, Human kinetic adjustment factor, Interindividual variability factor, Monte Carlo simulations, Population variability, Risk assessment, Steady-state toxicokinetics

LIST OF ABBREVIATIONS AND ACRONYMS

CSAF	**chemical-specific adjustment factor**
CAss	**arterial blood concentration at steady-state**
CYP	**cytochrome P-450**
HKAF	**human kinetic adjustment factor**
$HKAF_{(ad/pop)}$	**human kinetic adjustment factor using as referent the median in either the adults (ad) or in the population (pop)**
IVF	**interindividual variability uncertainty factor**
Km	**Michaelis-Menten constant**
P_b	**blood:air partition coefficient**
PBPK	**physiologically-based pharmacokinetic**
POD	**point of departure**
Ql	**liver blood flow**
Qp	**alveolar ventilation rate**
RAM	**rate of metabolism**
RfC	**reference concentration**
RfD	**reference dose**
Vmax	**maximum rate of metabolism**
Vl	**volume of liver**
VOC	**volatile organic compound**

6.1 Introduction

An default interindividual variability (or uncertainty) factor (IVF) of a default value of 10 is usually applied to the point of departure (POD) for deriving reference doses (RfD) or reference concentrations (RfC) for use in non-cancer risk assessment (Dourson *et al.*, 1996; Dourson and Stara, 1983; U.S. EPA, 2002). As reviewed by Price *et al.* (1999), the IVF has historically been defined as a factor required to protect the sensitive members of the population since the POD is generally determined for average healthy individuals. Actually, two models have been proposed to describe the IVF. Under the "sensitive population" model, the IVF is applied to correct for the possible failure of a critical study to include a sufficient number of members pertaining to distinct subpopulation exhibiting an increased sensitivity. Conversely, under the "finite sample size" model, the application of the IVF relates to the possibility that the retained POD may fail to identify the toxicity threshold in the overall population simply because of the finite size of the sample in which it was determined (Price *et al.*, 1999). Thus, the IVF accounts for the overall biological variability in the human population.

In the last 20 years, the IVF has been divided into two constitutive components (toxicokinetic and toxicodynamic factors), equal to 3.16 each based on pharmaceutical data (Dorne and Renwick 2005; IPCS, 1994; Renwick and Lazarus, 1998). This subdivision can be used in the evaluation of the magnitude and adequacy of the IVF for specific chemicals, and its replacement when appropriate data are available, by quantifying chemical-specific adjustment factors (CSAFs) (IPCS, 2005; Meek *et al.*, 2002). Under this method, the CSAF for interindividual variability in toxicokinetics, also referred to as the human kinetic adjustment factor (HKAF), can be determined based on the population distributions of relevant pharmacokinetic parameters (e.g., half-life, maximal concentration). The HKAF is calculated as the ratio between the upper percentile value of a parameter (*i.e.*, 95^{th}) and its central tendency value (*i.e.*, median) in the whole population or between an upper

percentile value in a presumed susceptible subpopulation and the central tendency value in the general healthy population (IPCS, 2005; Meek *et al.*, 2002).

Neither the historical definitions of IVF (Price *et al.*, 1999) nor the IPCS guidance document on CSAFs (IPCS, 2005) clearly define the “average healthy individual”, forming the point of comparison for the presumed sensitive subpopulations. Particularly, it is unclear as to whether this individual is the average healthy adult or the average healthy individual from the whole population (which includes both healthy adults and sensitive subpopulations). But presumably because the POD used to derive the RfD or RfC is generally determined in healthy adults (animal or human) (U.S. EPA, 2010), HKAF evaluations conducted using experimental data for drugs (Ginsberg *et al.*, 2002; Dorne *et al.*, 2005; Dorne, 2007) or PBPK model simulation data for environmental toxicants (Gentry *et al.*, 2002, Pelekis *et al.*, 2001, 2003; Clewell *et al.*, 2004; Mörk and Johanson, 2010; Nong *et al.*, 2006; Valcke and Krishnan, 2011a, 2011b) have relied on what can be called a "distinct subpopulation" approach. Thus, the experimental or simulated data in the presumed susceptible individuals (e.g. neonates, pregnant women, elderly, polymorphic individuals) have often been compared with the corresponding data in healthy adults.

Alternatively, HKAF can be quantified using a "whole population" approach as done recently by Mörk and Johanson (2010). In this study, HKAFs were calculated for inhaled acetone based on a simulated distribution of steady-state blood concentration in an entire population, including adults and various age-defined groups of children. The PBPK modeling results in the different subgroups were weighted according to demographic representation in Sweden. Excluding the endogenous production of acetone, an HKAF of 1.9 was obtained by dividing the 95^{th} percentile value of the entire population by the median. In comparison, using the 95^{th} percentile value of that same dose metric in 3-month-old babies as well as 10 and 15 yr old children resulted in HKAFs of 2, 2.4 and 1.7, respectively.

The hypothesis that the HKAF determined upon the “whole population” approach is quite different from the one determined based on the “distinct subpopulation” approach can be generated from the results of Mörk and Johanson (2010). This potential difference could be significant from a regulatory standpoint because it may not lead to comparable levels of protection for the different subgroups that compose the whole population. It is also not known whether the population composition and the chemical considered may impact this potential difference. Thus, the objective of the current study was to evaluate the magnitude and adequacy of the HKAFs determined by the "whole population" approach as compared to the "distinct subpopulation" approach. In effect, population distributions of internal dose metrics following chronic exposure to two chemicals exhibiting different clearance characteristics were used to compute the HKAF as:

- the ratio of the upper percentile value in the entire population including adults and non-adults over the median in adults and in this entire population;

- the ratio of the upper percentile value in presumed susceptible subpopulation over the median in adults and in the entire population including adults and non-adults.

6.2 Methods

A physiologically based steady-state algorithm combined with Monte Carlo simulation software was used to generate population distributions of blood concentration (CAss) and rate of metabolism (RAM) for chronic inhalation exposure to two chemicals with contrasting systemic clearance characteristics. The population distributions were reconstructed based on different proportions of randomly selected adults, elderly, children, neonates and PW, and they were used to compute HKAFs based on "whole population" and "distinct subpopulation" approaches.

6.2.1 Selection of surrogate chemicals and their specific parameters

Two VOCs were chosen as surrogate chemicals because they exhibit contrasting systemic clearances based on their pulmonary clearance potential (different blood:air partition coefficient (P_b)) and their hepatic clearance (different hepatic extraction ratios). Benzene was chosen as an extensively cleared chemical because of its high pulmonary clearance (low P_b, 7.4) and high hepatic extraction ratio. Conversely, 1,4-dioxane was chosen as a poorly cleared chemical due to its low pulmonary clearance (P_b = 3650) and low hepatic extraction ratio. While benzene is a known substrate of CYP2E1 (Ronis *et al.* 1996), for which extensive data on interindividual variability are available (Johnsrud *et al.* 2003; Lipscomb *et al.*, 2003), 1,4-dioxane was included in this study to facilitate the coverage of a range of physico/biochemical properties of potential substrates of CYP2E1 (Nannelli *et al*. 2005). Chemical-specific parameters are indicated in Table 6-I and were taken from the literature (Haddad *et al.* 2001; Reitz *et al.* 1990, U.S. EPA, 2010). The choice of these two surrogate VOCs and associated kinetic parameters was undertaken to reflect the range of kinetic characteristics of hypothetical substances for evaluating the HKAF. As such, the present modeling study did not focus on any aspect of the risk assessment relating to these specific chemicals.

6.2.2 Use of a biologically-based steady-state model for the simulation of continuous inhalation exposure in different subpopulations

The current study relies on the use of a steady-state model for inhalation exposures (e.g. Andersen, 1981; Chiu and White 2006; Csanady and Filser, 2001; Pelekis *et al.*, 1997) because the current study aimed at reconstructing population distributions of internal dose metrics for continuous lifetime exposures. Briefly, the algorithm computes the arterial blood concentration at steady-state (CAss) from the alveolar ventilation rate (Qp), the

concentration in air (Ci) and the hepatic ($Ql \times E_{hep}$) and pulmonary (Qp / Pb) clearances (Pelekis *et al.*, 1997):

$$CAss = \frac{Qp \times Ci}{Ql \times E_{hep} + Qp/P_b} \quad (1)$$

… where Ql is the liver blood flow, Pb is the blood:air partition coefficient and E_{hep} is the hepatic extraction ratio of the chemical and is calculated from its intrinsic clearance (Clint) as follows:

$$E_{hep} = \frac{Clint}{Clint + Ql} \quad (2)$$

Also, the rate of metabolised parent compound per unit volume of liver (RAM) is calculated as:

$$RAM = \frac{CA_{ss} \times Ql \times E_{hep}}{Vl} \quad (3)$$

As indicated in Table 6-II, Qp, Ql and Vl were calculated for a given individual by applying equations derived from Price *et al.* (2003a) to the individual's body weight (Valcke and Krishnan, 2011b). The input data are listed in Table 6-II for each subpopulation considered (Price *et al.* 2003a, Johnsrud *et al.*, 2003; ICRP, 2002; Lipscomb *et al.*, 2003; Valcke and Krishnan, 2011a, 2011b). These include six age groups covering the lifespan (neonates (0–30 d), infants (1–12 mo), toddlers (1–3 yr), children/adolescents (4–17 yr), adults (18–64 yr), and elderly (65–90 yr)), as well as pregnant women (15–44 yr). Ql and Vl for pregnant women were actually calculated on the basis of the body weight

for non-pregnant women, whereas the appropriate increase in alveolar ventilation rate at any time during pregnancy was accounted for when computing Qp (Valcke and Krishnan, 2011b).

6.2.3 Generation of distributions of the internal dose metrics by means of Monte Carlo simulations

Constant inhalation exposure to a benzene concentration corresponding to 10 × the RfC (Table 6-I) was simulated in each subpopulation. Given the lack of an RfC for 1,4-dioxane and its approximately ten-fold greater RfD compared to benzene (U.S. EPA, 2010), a concentration that was ten times greater than the benzene concentration was specified. Monte Carlo simulations were performed using the Crystal Ball® software (Oracle™, Redwood Shores, CA) to generate distributions of the various internal dose metrics (see below). The intrinsic clearance in Eq. 2 was corrected for a given individual in any subpopulation by adjusting the maximum rate of metabolism ($Vmax_{ind}$) using enzyme-specific catalytic turnover (Nong *et al.*, 2006; Valcke and Krishnan, 2011a, 2011b). This was determined based on the Vmax in an adult of average body weight (BW_{avg_ad}), as well as the [individual (ind)/average adult (avg_ad)] ratios of the liver volumes and CYP2E1 hepatic content (in pmol/mg of microsomal protein):

$$Vmax_{ind} = \frac{Vmaxc \times BW_{avg\_ad}^{0.75}}{[CYP2E1]_{avg\_ad} \times Vl_{avg\_ad}} \times [CYP2E1]_{ind} \times Vl_{ind} \quad (4)$$

A constant hepatic microsomal protein concentration was assumed across the subpopulations as discussed in Valcke and Krishnan (2011b).

6.2.3.1 Distributions in the "whole population" and corresponding HKAFs

Distributions of the internal dose metrics were generated for a theoretical population of 100,000 people with the demographic characteristics of Canada (Statistiques Canada, 2010). Therefore, the number of iterations used in the Monte Carlo simulations for each subpopulation corresponded to the targeted number of individuals. This number was based on the demographic proportions of each subpopulation (Table 6-III). Because the number of individuals appeared relatively constant across census' age ranges of same duration, the number of individuals pertaining to an age range different than those defined in the census could easily be estimated. For example, the number of toddlers aged 1–3 was considered as 60 % of the total individuals 0–4 yr old. Finally, the number of pregnant women was calculated based on the pregnancy rate of 104/1,000 from Ventura *et al.* (2008) and on the number of women aged 15–44 yr from the census data. The dose metric values "generated" by the Monte Carlo simulations for each subpopulation were then merged into a single "Canadian population dataset" of 100,000 values. To calculate the HKAFs based on the "whole population" approach, the ratio of the upper percentile value of the dose metric in the entire Canadian population to its median value was computed. The percentage of each subpopulation that was protected by a "whole population" HKAF was determined by identifying the number of individuals in each subpopulation exhibiting an internal dose metric that was lower than the entire population's upper percentile value underlying the HKAF, *i.e.* 95^{th} or 99^{th}.

6.2.3.2 Distributions in each "distinct subpopulation" and corresponding HKAFs

Distributions of the internal dose metrics for 100,000 individuals of each subpopulation were generated and the chemical- and dose metric-specific HKAFs were calculated based on the "distinct subpopulation" approach, i.e. as the ratio of the upper percentile value (*i.e.*, 95^{th} or 99^{th}) in each subpopulation to the median value in adults or the whole Canadian population (see above). Also, for a given dose metric, the greatest "distinct subpopulation"-based HKAF was multiplied by the median in the whole Canadian population (see above)

to obtain a threshold dose metric value. This threshold corresponded to the percentile that was referred to for determining the proportion of individuals from the entire population that was covered by the greatest "distinct subpopulation" HKAF.

6.2.4 Evaluation of the impact of demography on the computed HKAFs

Given that the HKAF values as computed herein rely on the distribution of internal dose metrics in a general population composed of various proportions of each subpopulation, it was hypothesized that the demographic characteristics of a given general population may impact this calculation. To test this hypothesis, HKAFs were evaluated on the basis of dose metric distributions generated for a theoretical "younger population". These distributions were reconstructed by multiplying by 3 the number of individuals of each subpopulation < 18 yr, as well as pregnant women, as compared to the numbers that were previously used to reconstruct the Canadian population distributions(Table III). The number of adults was also adjusted to maintain a total of 100,000 dose metric values. Thus, more than 60% of the resulting "younger population" was aged < 18 y, as compared to approximately 20% for the Canadian population.

6.3 Results

6.3.1 Distributions of internal dose metrics in each subpopulation and the entire Canadian population.

Figs. 6.1 and 6.2 show simulation of the internal dose metric distributions in each subpopulation (making up the entire Canadian population) exposed to benzene and 1,4-dioxane, respectively. The shapes of the Canadian population distributions appeared normal for CAss of benzene and lognormal in the other cases. The ranges (1^{st}–99^{th} percentile) and median dose metrics that were obtained when simulating 100,000 individuals in each

subpopulation are indicated in Table 6-IV. Based on the median and 99th percentile dose metrics, neonates and pregnant women were the most susceptible subpopulations (*i.e.* they exhibited the highest dose metric) based on CAss and RAM, respectively. The median dose metric in the most susceptible subpopulation was always greater than the median dose metric in the Canadian population, but it was lower than the 99th percentile value, except for the CAss value for 1,4-dioxane. In this case, the median value in neonates (2.3 mg/L) was greater than the 99th percentile value in the whole population (2.14 mg/L). The internal variability of internal dose metrics in the Canadian population can be appaised by the ratio of the 99th to the 1st percentile values. The greatest variability was obtained for 1,4-dioxane based on simulations of CAss exhibiting an approximately seven-fold difference. The population variability was lower in every other case ([99th/1st percentile] ratios lower than 3). Similar trends were obtained for each specific subpopulation, although the magnitude of the differences varied. In particular, neonates exhibited a ten-fold [99th/1st percentile] ratio of CAss for 1,4-dioxane. This dose metric exhibits a variability leading to such ratio that is always greater than 5 regardless of the subpopulation. In every other subpopulation and dose metric, the ratio was at most equal to 3 (neonates' RAM for 1,4-dioxane).

6.3.2 HKAF values

6.3.2.1 "Whole population" approach

The HKAFs determined based on the "whole population" approach, which used both the median adult ($HKAF_{ad}$) and the median individual in the entire Canadian population ($HKAF_{pop}$) as referents, are indicated in Table V. CAss-based HKAFs varied between 1.2 and 1.3 for benzene and between 2.1 and 2.8 for 1,4-dioxane. These values were slightly lower than the highest "distinct subpopulation"-based HKAFs for benzene but were significantly lower than the 1,4-dioxane values (see below). Considering the RAM, all the HKAF values were between 1.2 and 1.6 regardless of the chemical. These values were slightly lower than the highest RAM-based HKAFs obtained with the "distinct subpopulation" approach in pregnant women (1.5–2.1, see below).

6.3.2.2 "Distinct subpopulation" approach

Table 6-V shows that the 95th and the 99th percentile-based HKAFs that were computed using the "distinct subpopulation" approach were comparable whether the median adult ($HKAF_{ad}$) or the median individual in the whole population ($HKAF_{pop}$) was used as a referent. In addition to the referent adults, results for neonates and pregnant women are presented because they were, toxicokinetically, the most susceptible based on their respective CAss and RAM (Table 6-IV). HKAFs for infants were also shown because they exceeded the default 3.16 value when CAss of 1,4-dioxane was considered, on the basis of the 99th percentile value (3.8). The default value was also exceeded based only on CAss of 1,4-dioxane in neonates (range: 6.5–8.5) and the 99th percentile value in pregnant women (3.5). Neonates exhibited the greatest CAss-based HKAFs for inhaled benzene (1.6–1.7). Otherwise, pregnant women showed the greatest RAM-based HKAFs for benzene (1.5–1.6) and 1,4-dioxane (1.8–2.1). HKAFs in other subpopulations remained in the range of the HKAFs presented in Table 6-V for any given dose metric (data not shown).

6.3.3 Proportions of the whole population or the distinct subpopulations covered by the different HKAFs

Table 6-VI shows the proportion of each subpopulation that was covered by the various HKAFs defined using the "whole population" approach. The 95th or 99th percentile-based "whole population" HKAFs generally protect at least, or very close to, 95 % and 99 %, respectively, of the individuals of each subpopulation. However, only 57 % of the neonates, 78 % of the pregnant women and 89 % of the infants were covered by the 95th percentile-based "whole population", CAss-based HKAF for benzene. Corresponding values for the 99th percentile-based HKAF values were 73 %, 92 % and 97 %, respectively. In the case of 1,4-dioxane, 27 %, 76 % and 86 % of the neonates, infants, and pregnant women were covered by the 95th percentile-based HKAF, respectively. Corresponding values for the 99th

percentile HKAFs were 48 %, 92 %, and 96 %, respectively, and the default 3.16 factor appears to cover only 60 % of the neonates. Considering the RAM, the lack of coverage by the "whole population"-based HKAF concerns pregnant women, as only 63 % and 85 % of them are covered by respectively the 95^{th} and 99^{th} percentile-based HKAF for benzene. These numbers are 66 % and 86 % in the case of 1,4-dioxane. Finally, when the HKAF was computed with the "distinct subpopulation" approach and the greatest value was retained, more than 99 % of the entire Canadian population was covered by every dose metric considered (Table 6-VI).

6.3.4 Impact of the demography on the computed HKAFs

The impact of the demographic characteristics on the HKAF values as computed herein can be appreciated from the results shown in Fig. 6.3 for CAss and Fig. 6.4 for RAM. The distributions for 100,000 referents (adult) and the most susceptible individuals (neonates for CAss, pregnant women for RAM) are also shown in these figures for comparison purposes. For benzene (Fig. 6.3a), the change in demographics did not impact the overall population distribution of CAss (indistinguishable from adults) and thus, not the HKAF. The change of demographics shifts minimally to the right the population distribution of CAss for 1,4-dioxane (Fig. 6.3b). The impact on the various HKAFs was low ("whole population" HKAF = 2.69 *vs.* 2.75) with differences of 3 % or less for the relevant statistical descriptors. Considering RAM, when the number of significantly less susceptible neonates, and infants, and more susceptible pregnant women, was increased at the expense of adults, the entire population distribution of the dose metric for both chemicals was widened slightly and particularly for 1,4-dioxane (Fig. 6.4). The impact of pregnant women was apparent with a slight burst in the "younger" population distribution, which was observed near the pregnant women's approximate median value. On the basis of the 99^{th} percentile values, this resulted in virtually unchanged HKAFs for benzene (Fig. 6.4a), but marginal changes were observed for 1,4-dioxane (Fig. 6.4b). The "whole population" HKAFs that were calculated from the indicated statistical descriptors are 1.67 (1342/806)

and 1.75 (1342/765), which were based on median values in adults or population distributions, respectively, for the "younger" population. In comparison, the "whole population" HKAF for the Canadian population was 1.62. The "distinct subpopulation" HKAF for pregnant women was also slightly increased, from 2.09 for the Canadian and adult populations to 2.2 for the "younger" population due to lower median values (765 µg/h-L of liver *vs.* 808 or 806 µg/h-L of liver).

6.4 Discussion

This study performed Monte Carlo simulations on a steady-state algorithm to reconstruct representative subpopulations and whole population distributions of internal dose metrics for continuous inhalation exposure to a highly (benzene) and poorly (1,4-dioxane) cleared chemical. This allowed evaluating the impact of various assumptions on the resulting HKAF.

Virtual populations have been reconstructed to evaluate the population variability of the pharmacokinetic of drugs (e.g.: Jamei *et al.*, 2009; Hudachek and Gustafson, 2011), but to date, the same approach had not been realized for environmental contaminants. This procedure realized in the context of the present study allowed obtaining results showing that the impact of the approach chosen to compute the HKAF depends on the chemical and dose metric considered. The “whole population” approach used here can be related to the “Finite Sample Size” model of IVF from Price *et al.* (1999), whereas the "distinct subpopulation"-based HKAF can be associated to these authors’ “sensitive population” model. When the most sensitive individuals, based on dosimetric considerations, constitute a very small fraction of the entire population, a "whole population"-based HKAF might not be sufficient to cover them adequately. For instance, less than 60 % of the neonates, constituting less than 0.1 % of the whole Canadian population in this study, were covered by the "whole population" HKAF based on their 95th percentile CAss value. This was also

for the case of infants, who constituted a mere 1 % of the entire population, for whom less than 90 % of the individuals simulated were covered by that same HKAF (Table 6-VI). The reasons for these results can be determined from Figs. 6.1 and 6.2. Because toxicokinetically sensitive neonates and infants make up a small proportion of the population, their CAss values do not stand out at the right end of the whole population distributions (Figs. 6.1a and 6.2a). Thus, the "distinct subpopulation"-based HKAF would appear to be more adequate in these cases because the focus is then put on the most sensitive subpopulations, regardless of whether the data follow unimodal or bimodal distributions. Conversely, when the more sparse individuals (neonates and infants) are rather less sensitive than the vast majority of the individuals composing the entire population, as for RAM, the approach taken to compute the HKAF does not impact its value (Table 6-V).

The results obtained in Figs. 6.3 and 6.4 can be viewed as a “sensitivity analysis” of the impact of demography on the HKAF. Replacing a significant number of adults from the Canadian population with individuals who are generally equally sensitive as adults (Table 6-IV) resulted in a "younger" population distribution of CAss for benzene that remained virtually unchanged (Fig. 6.3a). In the case of 1,4-dioxane (Fig. 6.3b), every replacing individual pertained to subpopulations that were more susceptible than adults (Table 6-IV), and, as a result, the population distribution of CAss slightly shifted to the right towards the most sensitive neonates. In the case of RAM, the individuals replacing the adultes were either more susceptible (pregnant women) or less susceptible (children), leading to population distributions that were wider for both chemicals (Fig. 6.4). As mentioned in the *Results* section, the sensitivity of the HKAF to the population demography (i.e. the impact of the population distribution shift on the estimated HKAF) was marginal because the differences in the susceptibilities were not very pronounced between the subpopulations, with the exceptions of neonates and infants based on CAss (particularly for 1,4-dioxane), and pregnant women based on the RAM. However, the

impact of these subpopulations' dose metric on the entire population distribution always remained minimal because of their small percentage in the entire population.

While in our study, demography appears to have, at the most, a very marginal effect on HKAF, the population distribution of CAss is significantly influenced by the determining physiological parameters. Indeed, intake and pulmonary clearance of both benzene and 1,4-dioxane are driven by alveolar ventilation rate, which is rather log-normally distributed when adjusted to the body weight (Fig. 6.5a). However, blood-flow limited metabolism results in hepatic blood flow being the determining parameter for the clearance of benzene whereas for 1,4-dioxane, hepatic enzyme concentration, and thus Vmax (see Eq. 4) is determinant of its enzyme-limited clearance. As a result, the distribution of body-weight adjusted liver blood flow (Fig. 6.5b), which is more skewed than the distributions of Vmax (Fig. 6.5c) or Clint (central tendency, range: ≈ 400 L/h, 0 - 1600 L/h), yields a population distribution of CAss that is more skewed for benzene (Fig. 6.1a) than for 1,4-dioxane (Fig. 6.2a). Indeed, the hepatic clearance of the latter is rather driven by the more widely distributed Vmax (Fig. 6.5d) and the corresponding Clint (central tendency, range: ≈ 1.7 L/h, 0 - 7 L/h). Correspondingly, "whole population"-based HKAFs are smaller for benzene than 1,4-dioxane (Table 6-V).

The toxicokinetic determinants, including the physiological paramters, of the susceptibility of each subpopulation to a given chemical based on any dose metric have been thoroughly discussed elsewhere (Valcke and Krishnan, 2011b). Briefly, neonates are the most susceptible population based on CAss (Table 6-V) because they are exposed to a greater-than-adult body weight-adjusted dose by inhalation, or to a poorly metabolized chemical (1,4-dioxane) for which hepatic metabolism is enzyme-limited, thus reduced in neonates. For pregnant women, their greater susceptibility on the basis of RAM is due to their increase intake on a body weight basis (due to high Qp) combined with their efficient hepatic clearance, a combination that yields a high rate of conversion of inhaled parent

compound into metabolites. Greater inhalation uptake on a body weight basis, and corresponding blood concentration of inhaled VOCs, for young children and pregnant women as compared to adults, have been consistently documented and discussed in the literature (Brochu *et al.*, 2006, 2011; Fausman and Ribeiro, 1990; ICRP, 2002; Nong *et al.*, 2006; Price *et al.*, 2003b; Sarangapani *et al.*, 2003; Valcke and Krishnan, 2011a, 2011b; WHO, 2006). The systemic clearance of high P_b, poorly metabolized 1,4-dioxane is Qp-dependent (for pulmonary clearance) and enzyme-dependent (for hepatic clearance), and the greater intra-subpopulation variability of CAss that was observed for this chemical was expected. This greater variability results in neonates and infants constituting the only subpopulation for which the consideration of the 99th percentile value rather than the 95th significantly changes their HKAF value (Table 6-V). Else, the intra-subpopulation variability was rather low for every dose metric.

The "distinct subpopulation"-based $HKAF_{ad}$ that was obtained for benzene exposure in infants (1.3–1.4, Table 6-V) and toddlers (1.25–1.33, not shown) were very similar to the values obtained for other inhaled VOCs by Pelekis *et al.* (2001) for a 10 kg child. Using a deterministic steady-state approach, these authors obtained an average factor of 1.1 ± 0.6 for eight chemicals highly cleared by either pulmonary or hepatic clearance or both. Also, a ratio of the neonate's 95th percentile value to the adult's median value of blood concentrations for dichloromethane was slightly above 2 in the study by Pelekis *et al.* (2003) for continuous inhalation exposure, as compared to 1.6 for benzene in the current study. Besides, the "distinct subpopulation"-based $HKAF_{pop}$ that was obtained in neonates for 1,4-dioxane (6.5, Table 6-V) was markedly greater than the value (2) obtained by Mörk and Johanson (2010) for acetone, a chemical that is similar to 1,4-dioxane (highly water soluble with a P_b of 260). The HKAF obtained for 1,4-dioxane in children and adolescents (1.8, not shown) is comparable to those obtained by these authors for 10 and 15 yr-old children for acetone (1.7 - 2.4). The discrepancy for neonates might be explained by the difference in the mean age considered (14 days *vs.* 3 months) and related hepatic enzyme content. The "whole population"-based $HKAF_{pop}$ obtained here based on the 95th percentile

(2.1) compares well to Mörk and Johanson's results (i.e. 1.9). Finally, Renwick and Lazarus (1998) determined that more than 99 % of individuals in a theoretical population of 1 million would be covered by the default factor, a proportion also obtained in this study.

Among the limitations of this study are other demographic characteristics, including gender differentiation that could have been considered when generating the population distributions. In particular, ethnicity can be a critical determinant of population variability in toxicokinetics (Renwick and Lazarus, 1998) because it is often linked to polymorphic metabolism (Haber *et al.*, 2002). However, multiplying subpopulation categories would increase the uncertainty linked to analyzing the distribution of the dose metric in very rare individuals like those with genetic polymorphisms. Besides, gender-related differences in the blood toxicokinetics of several VOCs have been considered insignificant (Clewell *et al.*, 2004; Sarangapani *et al.*, 2003). Furthermore, ethnicity is likely not a primary factor determining CYP2E1 activity because the population variability in the enzyme expression caused by factors other than polymorphism, such as ethanol consumption and xenobiotic coexposure (Neafsey *et al.*, 2009) is considerable. Another limitation relates to the use of only healthy individuals in this study; the HKAFs calculated in this study thus do not account for diseased people with altered hepatic or extrahepatic clearance.

In conclusion, this study has, for the first time, systematically compared different approaches for computing the HKAF under various assumptions related to the population/subpopulation variability in internal dose metric for continuous inhalation exposure. This was determined for two environmental chemicals exhibiting different patterns of systemic clearance, to encompass a range of other potential chemicals with such characteristics. This study contributes to clarify the implications of the different underlying assumptions that relate to the interindividual variability considered when determining the HKAF for any risk management consideration, including adequate coveriage or not of the most susceptible, but sparse, individuals of a given population. In this regard, relying on the

"distinct subpopulation" approach appears more conservative (protective) as it better covers the most susceptible individuals, in particular if they compose a small proportion of the general population. Fundamentally, the difference in the extent of coverage afforded by these two approaches would appear to depend upon the proportion of the most sensitive individuals in the target population for a risk assessment. Moreover, the present work has illustrated the feasibility of a novel approach for characterizing demography-based population variability of internal dose metrics for environmental contaminants.

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6.5 References

Andersen, M. E. 1981. Pharmacokinetics of inhaled gases and vapors. *Neurobehavioral. Toxicol. Teratol. 3*, 383-389.

Brochu, P., Ducré-Robitaille, J. F., Brodeur, J. 2006. Physiological daily inhalation rates for free-living pregnant and lactating adolescents and women aged 11 to 55 years, using data from doubly labelled water measurements for use in health risk assessment. *Hum Ecol Risk Assess 12*, 702-735.

Brochu, P., Brodeur, J., Krishnan, K. 2011. Derivation of physiological inhalation rates in children, adults, and elderly based on nighttime and daytime respiratory parameters. *Inhal. Toxicol. 23*, 74-94.

Chiu, W. A., White, P. 2006. Steady-state solutions to PBPK models and their applications to risk assessment I: Route-to-route extrapolation of volatile chemicals. *Risk Anal. 26*, 769-780.

Clewell, H. J., Gentry, P. R., Covington, T. R., Sarangapani, R., Teeguarden, J. G. 2004. Evaluation of the potential impact of age- and gender-specific pharmacokinetic differences on tissue dosimetry. *Toxicol Sci. 79*, 381-393.

Csanady, G. A., Filser, J. G. 2001. The relevance of physical activity for the kinetics of inhaled gaseous substances. *Arch. Toxicol. 74*, 663-672.

Dorne, J. L. 2007. Human variability in hepatic and renal elimination: implications for risk assessment. *J Appl. Toxicol. 27*, 411-420.

Dorne, J. L., Renwick, A. G. 2005. The refinement of uncertainty/safety factors in risk assessment by the incorporation of data on toxicokinetic variability in humans. *Toxicol. Sci. 86*, 20-26.

Dorne, J. L., Walton, K., Renwick, A. G. 2005. Human variability in xenobiotic metabolism and pathway-related uncertainty factors for chemical risk assessment: a review. *Food Chem. Toxicol. 43*, 203-216.

Dourson, M. L., Felter, S. P., Robinson, D. 1996. Evolution of science-based uncertainty factors in noncancer risk assessment. *Regul. Toxicol. Pharmacol. 24*, 108-120.

Dourson, M. L., Stara, J. F. 1983. Regulatory history and experimental support of uncertainty (safety) factors. *Regul. Toxicol. Pharmacol. 3*, 224-238.

Faustman, E. M., Ribeiro, P., 1990. *Pharmacokinetic consideration in developmental toxicity*, in: Hood, R.D. (Ed.), Developmental toxicology: risk assessment and the future. Van Nostrand Reinhold, New York, pp. 109-136.

Gentry, P. R., Hack, C. E., Haber, L., Maier, A., Clewell, H. J., III. 2002. An approach for the quantitative consideration of genetic polymorphism data in chemical risk assessment: examples with warfarin and parathion. *Toxicol. Sci. 70*, 120-139.

Ginsberg, G., Hattis, D., Sonawane, B., Russ, A., Banati, P., Kozlak, M., Smolenski, S., Goble, R. 2002. Evaluation of child/adult pharmacokinetic differences from a database derived from the therapeutic drug literature. *Toxicol. Sci. 66*, 185-200.

Haber, L. T., Maier, A., Gentry, P. R., Clewell, H. J., Dourson, M. L. 2002. Genetic polymorphisms in assessing interindividual variability in delivered dose. *Regul. Toxicol. Pharmacol. 35*, 177-197.

Haddad, S., Beliveau, M., Tardif, R., Krishnan, K. 2001. A PBPK modeling-based approach to account for interactions in the health risk assessment of chemical mixtures. *Toxicol. Sci. 63*, 125-131.

Hudachek, S. F., Gustafson, D. L. 2011. Customized in silico population mimics actual population in docetaxel population pharmacokinetic analysis. *J Pharm. Sci. 100*, 1156-1166

ICRP. 2002. Annals of the ICRP. ICRP Publication 89. *Ann ICRP. 32*, 5-265.

IPCS. 1994. Assessing human health risks of chemicals: Derivation of guidance values for health based exposure limits. 170. Geneva, World Health Organization, International Panel on Chemical Safety. Environmental Health Criteria.

IPCS. 2005. Chemical-Specific Adjustment Factors (CSAFs) for Interspecies Differences and Human Variability: Guidance Document for the Use of Data in Dose/ Concentration-Response Assessment. WHO. WHO/IPCS/01.4, 1-96. Geneva, Switzerland.

Jamei, M., Dickinson, G. L., Rostami-Hodjegan, A. 2009. A framework for assessing inter-individual variability in pharmacokinetics using virtual human populations and integrating general knowledge of physical chemistry, biology, anatomy, physiology and genetics: A tale of 'bottom-up' vs 'top-down' recognition of covariates. *Drug Metab Pharmacokinet. 24*, 53-75.

Johnsrud, E. K., Koukouritaki, S. B., Divakaran, K., Brunengraber, L. L., Hines, R. N., McCarver, D. G. 2003. Human hepatic CYP2E1 expression during development. *J Pharmacol. Exp. Ther. 307*, 402-407.

Lipscomb, J. C., Teuschler, L. K., Swartout, J., Swan, G. E Snawder, J. E. 2003. Variance of microsomal protein and cytochrome P450 2E1 and 3A forms in adult human liver. *Toxicol. Mech. Meth. 13*, 45-51.

Meek, M. E., Renwick, A., Ohanian, E., Dourson, M., Lake, B., Naumann, B. D., Vu, V. 2002. Guidelines for application of chemical-specific adjustment factors in dose/concentration-response assessment. *Toxicology. 181-182*, 115-120.

Mörk, A. K., Johanson, G. 2010. Chemical-specific adjustment factors for intraspecies variability of acetone toxicokinetics using a probabilistic approach. *Toxicol Sci. 116*, 336-348.

Nannelli A, De RA, Longo V, Gervasi PG. (2005). Effects of dioxane on cytochrome P450 enzymes in liver, kidney, lung and nasal mucosa of rat. Arch. Toxicol. 79, 74-82.

Neafsey, P., Ginsberg, G., Hattis, D., Johns, D. O., Guyton, K. Z., Sonawane, B. 2009. Genetic Polymorphism in CYP2E1: Population Distribution of CYP2E1 Activity. *J Toxicol. Environ Health B. 12*, 362-388.

Nong, A., McCarver, D. G., Hines, R. N., Krishnan, K. 2006. Modeling interchild differences in pharmacokinetics on the basis of subject-specific data on physiology and hepatic CYP2E1 levels: a case study with toluene. *Toxicol. Appl. Pharmacol. 214*, 78-87.

Pelekis, M., Gephart, L. A., Lerman, S. E. 2001. Physiological-model-based derivation of the adult and child pharmacokinetic intraspecies uncertainty factors for volatile organic compounds. *Regul. Toxicol. Pharmacol. 33*, 12-20.

Pelekis, M., Krewski, D., Krishnan, K., 1997. Physiologically based algebraic expressions for predicting steady-state toxicokinetics of inhaled vapors. *Toxicol. Meth. 7*, 205-225.

Pelekis, M., Nicolich, M. J., Gauthier, J. S. 2003. Probabilistic framework for the estimation of the adult and child toxicokinetic intraspecies uncertainty factors. *Risk Anal. 23*, 1239-1255.

Price, P. S., Conolly, R. B., Chaisson, C. F., Gross, E. A., Young, J. S., Mathis, E. T., Tedder, D. R. 2003a. Modeling interindividual variation in physiological factors used in PBPK models of humans. *Crit Rev Toxicol. 33*, 469-503.

Price, K., Haddad, S., Krishnan, K. 2003b. Physiological modeling of age-specific changes in the pharmacokinetics of organic chemicals in children. *J Toxicol. Environ Health A. 66*, 417-433.

Price, P. S., Keenan, R. S., Schwab, B. 1999. Defining the interindividual (intraspecies) uncertainty factor. *Human Ecol. Risk Assess. 5*, 1023-1033.

Reitz, R. H., McCroskey, P. S., Park, C. N., Andersen, M. E., and Gargas, M. L. 1990. Development of a physiologically based pharmacokinetic model for risk assessment with 1,4-dioxane. *Toxicol Appl. Pharmacol. 105*, 37-54.

Renwick, A. G., Lazarus, N. R. 1998. Human variability and noncancer risk assessment--an analysis of the default uncertainty factor. *Regul. Toxicol. Pharmacol. 27*, 3-20.

Ronis, M. J. J., Lindros, K. O., Ingelman-Sundberg, M. 1996. The CYP2E family. In C. Ioannides (Ed.), *Cytochrome P450 Metabolic and Toxicological Aspects*, CRC PRess, Boca Raton FL, pp. 211-240.

Sarangapani, R., Gentry, P. R., Covington, T. R., Teeguarden, J. G., Clewell, H. J., III. 2003. Evaluation of the potential impact of age- and gender-specific lung morphology and ventilation rate on the dosimetry of vapors. *Inhal. Toxicol. 15*, 987-1016.

Statistiques Canada. 2010, Estimations de la population selon le sexe et le groupe d'âge au 1er juillet 2010, Canada. (in french) http://www.statcan.gc.ca/daily-quotidien/100929/t100929b4-fra.htm. Last access: December 22nd 2010.

U.S.EPA. 2002. A review of the reference dose and reference concentration process. Risk Assessment Forum. EPA/630/P-02/00F. Washington DC.

U.S.EPA. 2010. Integrated Risk Information System. U.S.Environmental Protection Agency . Last access: December 22nd 2010. Washington DC.

Valcke, M., Krishnan, K. 2011a. Evaluation of the impact of the exposure route on the human kinetic adjustment factor. *Regul. Toxicol Pharmacol. 59*, 258-269.

Valcke, M., Krishnan, K. 2011b. Evaluation of the Impact of physico-chemical and biochemical characteristics on the human kinetic adjustment factor for systemic toxicants. *Toxicology 286, 36-47*. doi:10.1016/j.tox.2011.05.003.

Ventura, S. J., Abma, J. C., Mosher, W. D., Henshaw, S. K. 2008. Estimated pregnancy rates by outcome for the United States, 1990-2004. *Natl. Vital Stat. Rep. 56*, 1-25.

WHO. 2006. Principles for evaluating health risks in children associated with exposure to chemicals. World Health Organization Press. *Environmental Health Criteria 237*. Geneva, Switzerland.

FIGURE CAPTIONS

Figure 6.1 : Distributions of individual values obtained CAss (A) and RAM (B) in each subpopulation within the whole Canadian population for constant inhalation exposure to benzene. From top to bottom, the distributions are shown for the entire Canadian population (——), adults (——), children and adolescents (— —), elderly (— · —), toddlers (— — -), pregnant women (- - - - -), infants (- - - - -) and neonates (indistinguishable).

Figure 6.2 : Distributions of individual values obtained CAss (A) and RAM (B) in each subpopulation within the whole Canadian population for constant inhalation (B) exposure to 1,4-dioxane. From top to bottom, the distributions are shown for the entire Canadian population (——), adults (——), children and adolescents (— —), elderly (— · —), toddlers (— — -), pregnant women (- - - - -), infants (- - - - -) and neonates (indistinguishable).

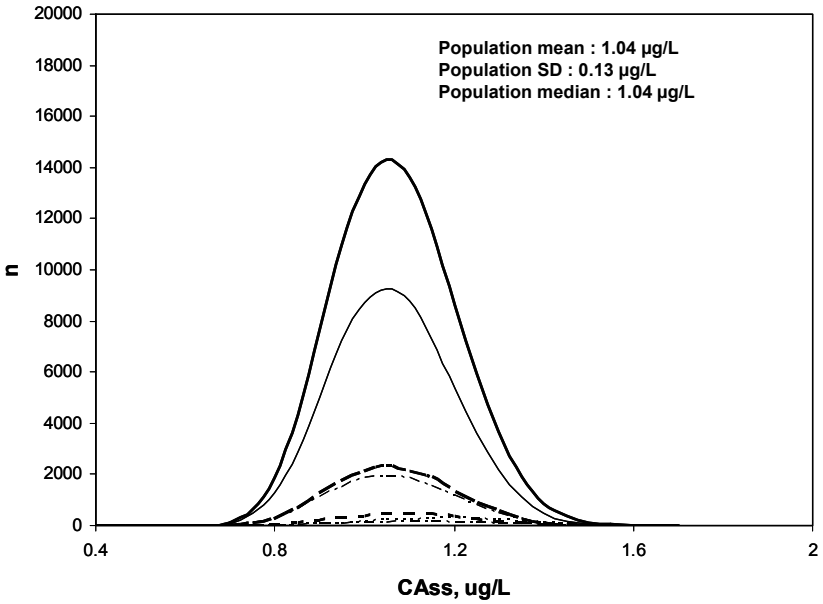
Figure 6.3 : Distribution of individual values obtained CAss for constant inhalation exposure to benzene (A) and 1,4-dioxane (B) in the entire Canadian (CP, ——) and "younger" (YP, — —) populations of 100,000 people, in 100,000 adults (——) and in 100,000 of the most susceptible neonates (neo, ...). Median and 99th percentile values only (for clarity reasons) are indicated.

Figure 6.4 : Distribution of individual values obtained RAM for constant inhalation exposure to benzene (A) and 1,4-dioxane (B) in the entire Canadian (CP, ——) and "younger" (YP, — —) populations of 100,000 people, in 100,000 adults (——) and in 100,000 of the most susceptible pregnant women (PW, ——).

Figure 6.5 : Distributions of individual values obtained for several physiological parameters in each subpopulation within the whole virtual Canadian population. From top to bottom, distributions for body weight-adjusted alveolar ventilation rate (A), body weight-adjusted liver blood flow (B) and maximal rate of metabolism of benzene (C) and 1,4-dioxane (D) are shown for the entire Canadian population (——), adults (——), children and adolescents (— —), elderly (– – –), toddlers (– – -), pregnant women (- - - - -), infants (- - - - -) and neonates (indistinguishable).

Figure 6.1

a)


Population mean : 1.04 µg/L
Population SD : 0.13 µg/L
Population median : 1.04 µg/L
n
CAss, ug/L
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
0.4
0.8
1.2
1.6
2

b)

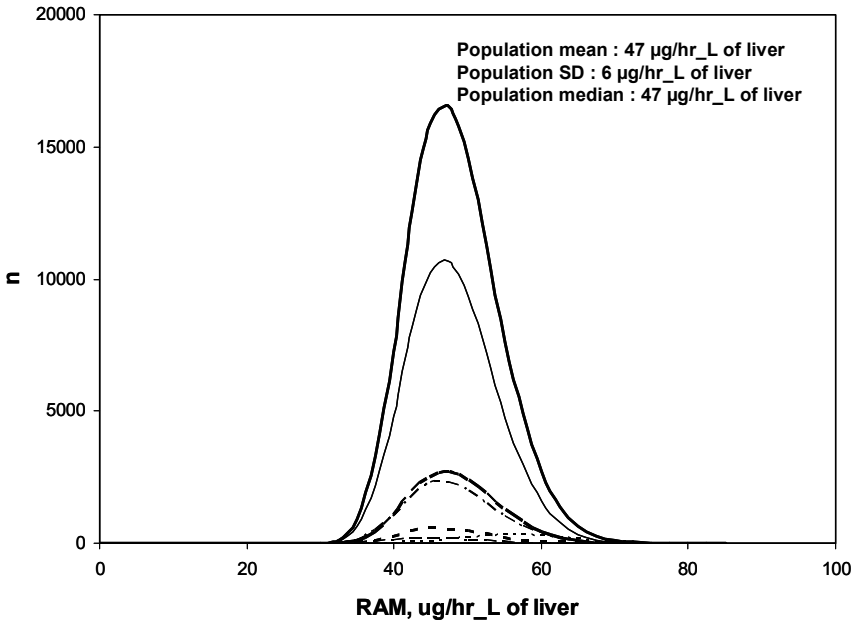
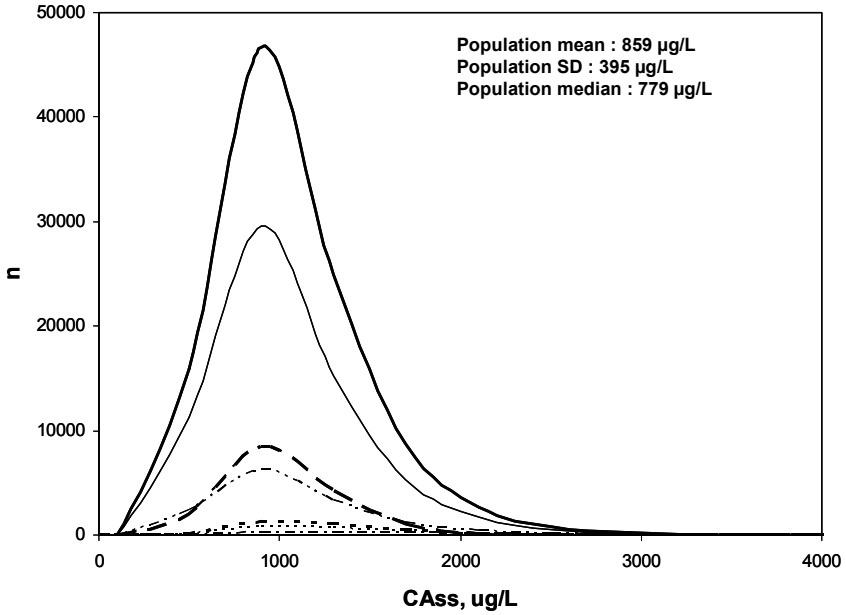

Population mean : 47 µg/hr_L of liver
Population SD : 6 µg/hr_L of liver
Population median : 47 µg/hr_L of liver
n
RAM, ug/hr_L of liver
20000
15000
10000
5000
0
0
20
40
60
80
100

Figure 6.2

a)


Population mean : 859 µg/L
Population SD : 395 µg/L
Population median : 779 µg/L
n
CAss, ug/L
0
10000
20000
30000
40000
50000
0
1000
2000
3000
4000

b)

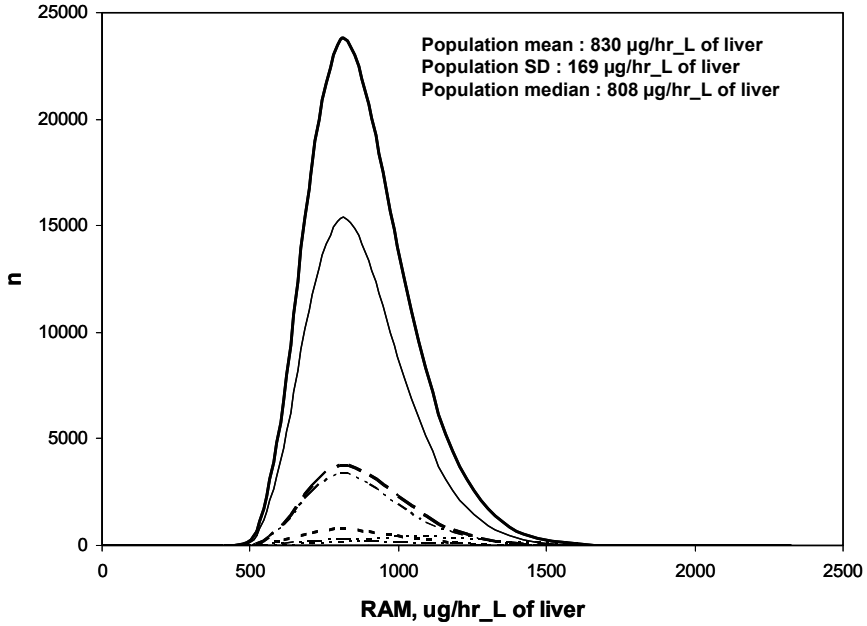
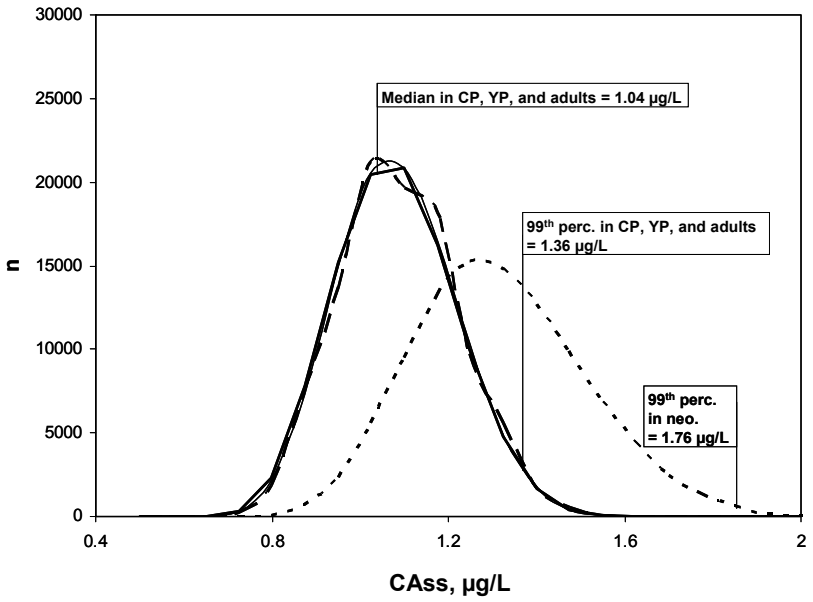

Population mean : 830 µg/hr_L of liver
Population SD : 169 µg/hr_L of liver
Population median : 808 µg/hr_L of liver
n
RAM, ug/hr_L of liver
0
5000
10000
15000
20000
25000
0
500
1000
1500
2000
2500

Figure 6.3

a)


30000
25000
20000
15000
10000
5000
0
n
0.4
0.8
1.2
1.6
2
CAss, µg/L
Median in CP, YP, and adults = 1.04 µg/L
99th perc. in CP, YP, and adults = 1.36 µg/L
99th perc. in neo. = 1.76 µg/L

b)

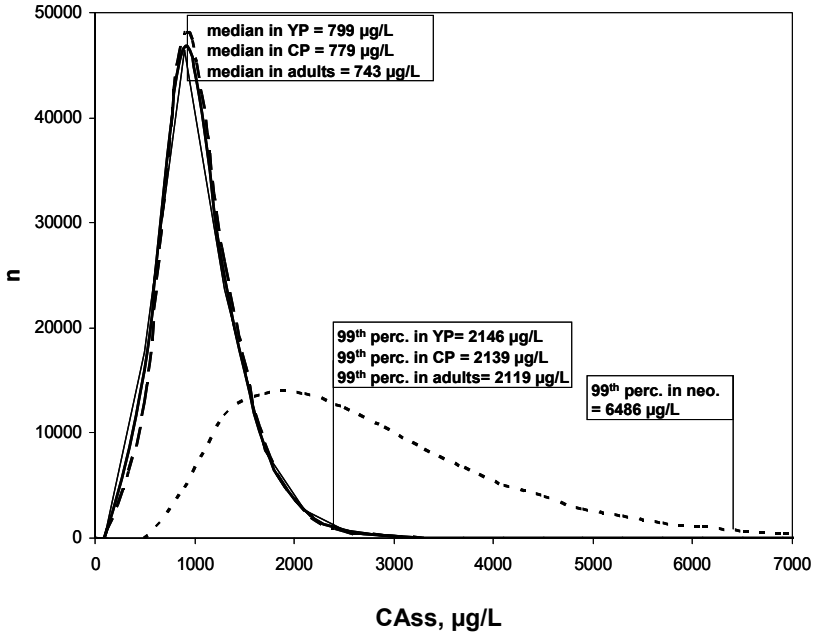
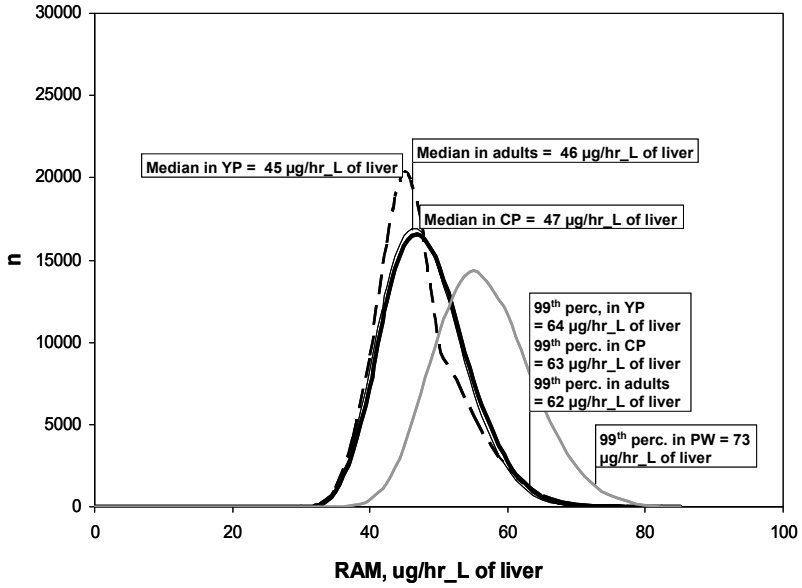

50000
40000
30000
20000
10000
0
n
0
1000
2000
3000
4000
5000
6000
7000
CAss, µg/L
median in YP = 799 µg/L
median in CP = 779 µg/L
median in adults = 743 µg/L
99th perc. in YP= 2146 µg/L
99th perc. in CP = 2139 µg/L
99th perc. in adults= 2119 µg/L
99th perc. in neo. = 6486 µg/L

Figure 6.4

a)


30000
25000
20000
15000
10000
5000
0
n
0
20
40
60
80
100
RAM, ug/hr_L of liver
Median in YP = 45 µg/hr_L of liver
Median in adults = 46 µg/hr_L of liver
Median in CP = 47 µg/hr_L of liver
99th perc, in YP = 64 µg/hr_L of liver
99th perc. in CP = 63 µg/hr_L of liver
99th perc. in adults = 62 µg/hr_L of liver
99th perc. in PW = 73 µg/hr_L of liver

b)

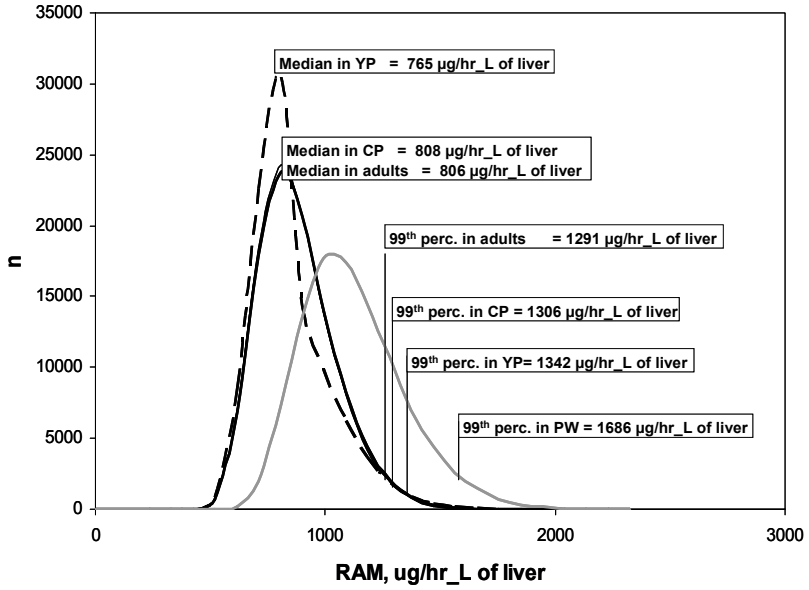
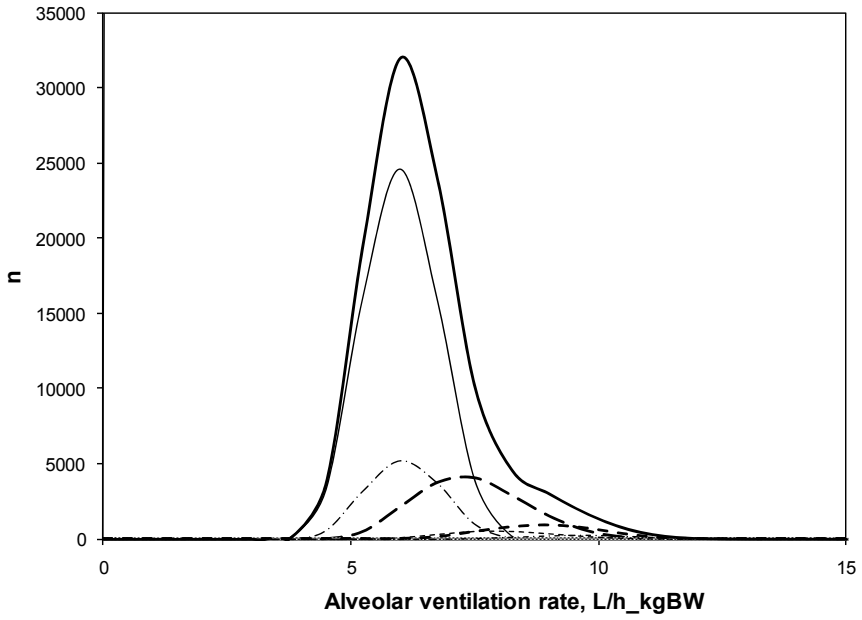

35000
30000
25000
20000
15000
10000
5000
0
n
0
1000
2000
3000
RAM, ug/hr_L of liver
Median in YP = 765 µg/hr_L of liver
Median in CP = 808 µg/hr_L of liver
Median in adults = 806 µg/hr_L of liver
99th perc. in adults = 1291 µg/hr_L of liver
99th perc. in CP = 1306 µg/hr_L of liver
99th perc. in YP= 1342 µg/hr_L of liver
99th perc. in PW = 1686 µg/hr_L of liver

Figure 6.5

a)


35000
30000
25000
20000
15000
10000
5000
0
n
0
5
10
15
Alveolar ventilation rate, L/h_kgBW

b)

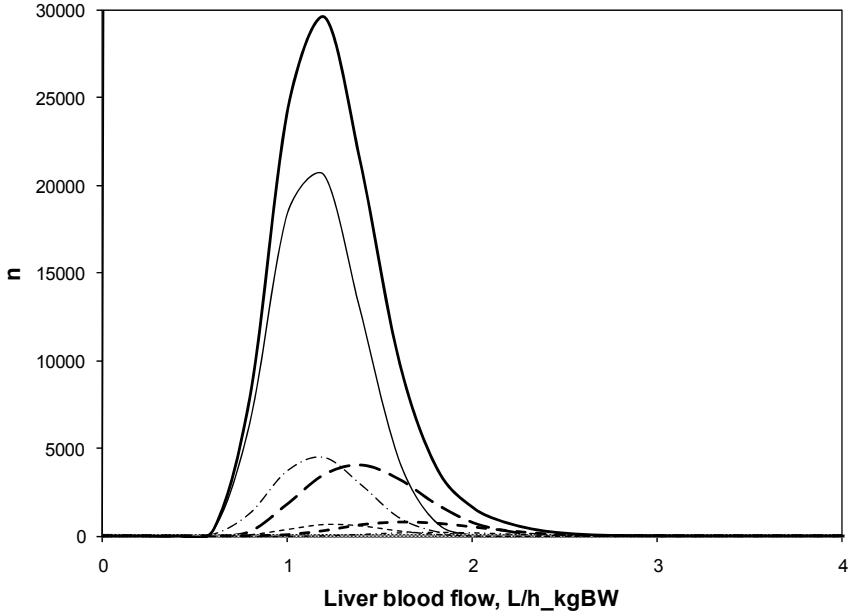
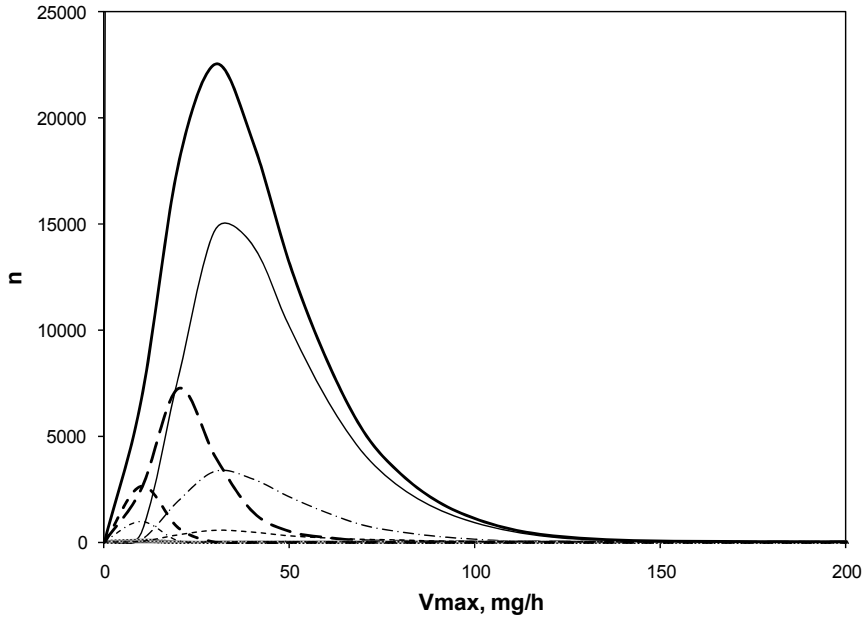

30000
25000
20000
15000
10000
5000
0
n
0
1
2
3
4
Liver blood flow, L/h_kgBW

Figure 6.5 (continued)

c)



d)

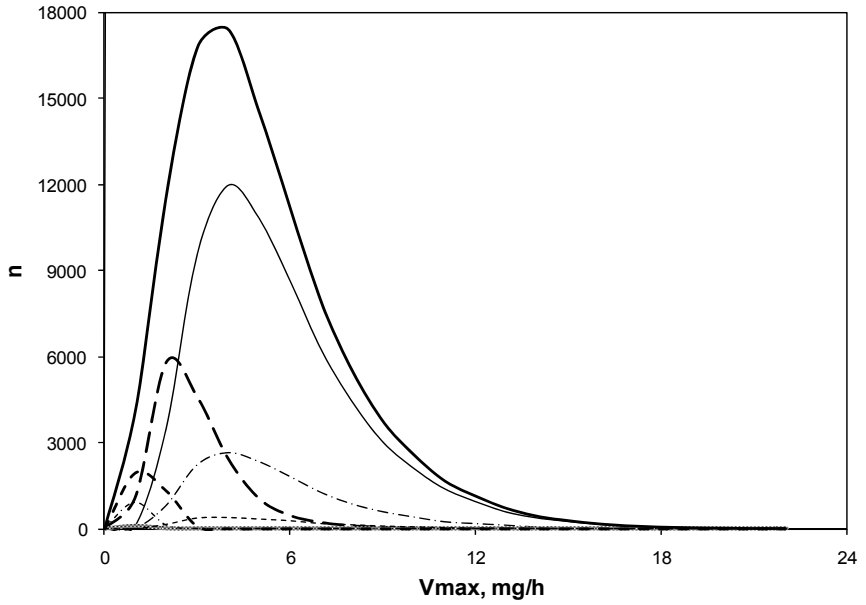


Table 6-I : Chemical-specific parameters used in the steady-state algorithm

Parameter	Chemical	
	Benzene[a)]	1,4-Dioxane[b)]
$Vmax_c$ (mg/h-kg$^{0.75}$)	2.11	0.27
Km (mg/L)	0.1	3.0
Blood:air partition coefficient (P_b)	7.4	3650
Exposure concentration (mg/m^3, UF × RfC)[c)]	0.3	3

a) Haddad *et al.*, 2001. b) Reitz *et al.*, 1990. c) U.S. EPA, 2010. Abbreviations: BW, body weight; Km, Michaëlis-Menten constant, P_b, blood:air partition coefficient, RfC, reference concentration; UF, interindividual uncertainty factor; $Vmax_c$, constant maximum rate of metabolism;

Table 6-II : Physiological parameter distributions used in the Monte Carlo simulations of the internal dose metrics with the steady-state algorithm

Subpopulation	Adults	Neonates	Infants	Toddlers	Children and adolescents	Elderly	Pregnant women
Median age (range)	41 (18–64)	14 d (0–30 d)	6.5 m (1–12 mo)	2 (1–3)	12 (4–17)	78 (65–90)	29 (15–44)
Parameter[a)]							
Sampled parameters							
Body weight (BW) (Kg: m ± SD, range)	76 ± 17, 37–152[b)]	4 ± 1, 2–7[c)]	9 ± 2, 3–15[b)]	13 ± 2, 7–32[b)]	36 ± 16, 9–113[b)]	72 ± 16, 33–155[b)]	70 ± 18, 36–152[d)]
[CYP2E1] (pmol/mg MSP: m ± SD, range)	49 ± 2, 11–130[e)]	18 ± 14, 1–56[c)]	36 ± 21, 10–86[c)]	42 ± 18, 18–74[c)]	53 ± 23, 22–95[c)]	f)	f)
Calculated parameters[b), g)]							
Alveolar ventilation rate (Qp)	= $[((0.2519 \times BW^{0.7609}) + (0.2508 \times BW^{0.7815})/2)]$ × « variability term » (*i.e.* 1 ± 0.1 (0.8–1.2))						
Liver volume (Vl)	= $0.05012 \times BW^{0.78}$ × « variability term » (*i.e.* 1 ± 0.14 (0.66–1.34))						
Liver blood flow (Ql)	= 0.92 × Vl × « variability term » (*i.e.* 1 ± 0.13 (0.67–1.33))						

a) Log normal distributions for sampled parameters and normal distributions for "variability terms". All indicated means are arithmetic, except note e) (see below). b) P^3M Database: Price *et al.* 2003. c) Johnsrud *et al.*, 2003. d) Distribution for non-pregnant women taken from P^3M database, and, to each of these values, the mean body weight increase at any week during pregnancy (normal distribution of 5 ± 4.4 kg (0-14.1)) based on data from ICRP was added (ICRP, 2002; Valcke and Krishnan, 2011b).. e) Lipscomb *et al.* (2003) (Geometric mean ± geometric standard deviation. MSP: microsomal protein.). f) Same as for adults. g) Valcke and Krishnan, 2011b. **Abbreviations**: CYP2E1, cytochrome p-450 2E1; MSP, microsomal protein; SD, standard deviation.

Table 6-III : Reconstruction of the hypothetical populations of 100,000 people with the Canadian demographic profile

Subpopulation (age range)	**Canadian Population in 2009[a)] Median age = 39.7 yr** Population size (%)	Corresponding reconstructed population size and number (n) of Monte Carlo iterations
Adults (18–64)	21,685,253 (63.92)	63,923
Neonates (0–30 d)	31,303 (0.09)	93
Infants (1–12 mo)	344,329 (1.02)	1,015
Toddlers (1–3)	1,126,896 (3.32)	3,322
Children and adolescents (4–17)	5,382,420 (15.87)	15,866
Elderly (65–90)	4,634,673 (13.66)	13,662
Pregnant women[b)] (15–44)	718,950 (2.12)	2,119
TOTAL	**33,923,824 (100)**	**100,000**

a) Statistiques Canada (2010) excluding the elderly aged > 90. b) Based on a pregnancy rate of 104/1,000 in U.S. women aged 15– 44 yr (Ventura *et al.*, 2008).

Table 6-IV : Distribution statistics of various dose metrics in each subpopulation based on 100,000 Monte Carlo iterations and the entire Canadian populations for constant inhalation exposure

Subpopulation / Dose metrics / Statistics	Benzene		1,4-Dioxane	
	CAss	RAM	CAss	RAM
Adults				
1st percentile	0.76	35	285	531
Median	1.04	46	763	806
99th percentile	1.36	62	2119	1291
Neonates				
1st percentile	0.88	20	682	371
Median	1.26	39	2299	686
99th percentile	1.76	55	6486	1149
Infants				
1st percentile	0.8	33	420	526
Median	1.11	45	1150	787
99th percentile	1.44	60	2928	1246
Toddlers				
1st percentile	0.79	35	382	534
Median	1.08	47	968	804
99th percentile	1.38	60	2099	1271
Children and adolescents				
1st percentile	0.77	35	352	538
Median	1.04	47	774	814
99th percentile	1.35	61	1744	1293
Elderly				
1st percentile	0.76	35	286	530
Median	1.04	46	766	807
99th percentile	1.37	62	2144	1291
Pregnant Women				
1st percentile	0.85	41	372	673
Median	1.16	55	995	1050
99th percentile	1.49	73	2698	1686
CANADIAN POPULATION				
1st percentile	0.76	35	299	533
Median	1.04	47	779	808
99th percentile	1.36	63	2139	1306

Symbols: CAss, blood concentration of parent compound (μg/L); RAM, rate of metabolism (μg/h-L of liver)

Table 6-V : HKAFs obtained by the "distinct subpopulation" approach on the basis of 100,000 Monte Carlo iterations in adults, neonates, infants and pregnant women, and by the "Whole population" approach for the Canadian population

HKAF assumption \ Dose metrics	Benzene		1,4-Dioxane	
	CAss	RAM	CAss	RAM
"Whole population" approach				
$HKAF_{ad}$ [a)]				
Based on 95th percentile	1.2	1.3	2.1	1.4
Based on 99th percentile	1.3	1.4	2.8	1.6
$HKAF_{pop}$ [b)]				
Based on 95th percentile	1.2	1.2	2.1	1.4
Based on 99th percentile	1.3	1.4	2.8	1.6
"Distinct subpopulation" approach				
In adults				
$HKAF_{ad}$ [c)]				
Based on 95th percentile	1.2	1.2	2.1	1.4
Based on 99th percentile	1.3	1.3	2.8	1.6
$HKAF_{pop}$ [d)]				
Based on 95th percentile	1.2	1.2	2.0	1.4
Based on 99th percentile	1.3	1.3	2.7	1.6
In neonates				
$HKAF_{ad}$ [c)]				
Based on 95th percentile	*1.6*	1.1	*6.6*	1.2
Based on 99th percentile	*1.7*	1.2	*8.5*	1.4
$HKAF_{pop}$ [d)]				
Based on 95th percentile	*1.6*	1.1	*6.5*	1.2
Based on 99th percentile	*1.7*	1.2	*8.3*	1.4
In infants				
$HKAF_{ad}$ [c)]				
Based on 95th percentile	1.3	1.2	3.1	1.4
Based on 99th percentile	1.4	1.3	3.8	1.6
$HKAF_{pop}$ [d)]				
Based on 95th percentile	1.3	1.2	3.0	1.4
Based on 99th percentile	1.4	1.3	3.8	1.5
In pregnant women				
$HKAF_{ad}$ [c)]				
Based on 95th percentile	1.4	*1.5*	2.7	*1.8*
Based on 99th percentile	1.5	*1.6*	3.5	*2.1*
$HKAF_{pop}$ [d)]				
Based on 95th percentile	1.4	*1.5*	2.6	*1.8*
Based on 99th percentile	1.5	*1.6*	3.5	*2.1*

Italicized values indicate the highest HKAF among each subpopulation for a given dose metric.

a) Computed as the ratio of the upper percentile value in the Canadian population (95^{th} or 99^{th}) to the median in 100,000 adults. b) Computed as the ratio of the upper percentile value in the Canadian population (95^{th} or 99^{th}) to its median. c) Computed as the ratio of the upper percentile value in the subpopulation (95^{th} or 99^{th}) to the median in adults. d) Computed as the ratio of the upper percentile value in the subpopulation (95^{th} or 99^{th}) to the median in the Canadian population.

Symbols: CAss, blood concentration of parent compound; $HKAF_{(ad/pop)}$, human kinetic adjustment factor using either the median in adult ("ad)" or whole population ("pop") as referent; RAM, rate of metabolism.

Table 6-VI : Percentage of individuals in the diverse Canadian subpopulations that are covered by the HKAF and the default factor for various dose metrics and chemicals

Dose metrics / Subpopulation / Variability descriptor	Benzene		1,4-Dioxane	
	CAss (%)	RAM (%)	CAss (%)	RAM (%)
Adults				
"whole population"[a] $HKAF_{95}^{th}$	96	96	95	97
"whole population"[a] $HKAF_{99}^{th}$	99	> 99	> 99	> 99
Default 3.16 factor	100	100	> 99	100
Neonates				
"whole population"[a] $HKAF_{95}^{th}$	57	100	27	100
"whole population"[a] $HKAF_{99}^{th}$	73	100	48	100
Default 3.16 factor	100	100	60	100
Infants				
"whole population"[a] $HKAF_{95}^{th}$	89	97	76	97
"whole population"[a] $HKAF_{99}^{th}$	97	> 99	92	> 99
Default 3.16 factor	100	100	97	100
Toddlers				
"whole population"[a] $HKAF_{95}^{th}$	93	97	92	96
"whole population"[a] $HKAF_{99}^{th}$	99	> 99	99	99
Default 3.16 factor	100	100	> 99	100
Children and adolescents				
"whole population"[a] $HKAF_{95}^{th}$	96	96	98	95
"whole population"[a] $HKAF_{99}^{th}$	> 99	> 99	> 99	> 99
Default 3.16 factor	100	100	100	100
Elderly				
"whole population"[a] $HKAF_{95}^{th}$	95	96	95	96
"whole population"[a] $HKAF_{99}^{th}$	> 99	99	99	> 99
Default 3.16 factor	100	100	> 99	100
Pregnant Women				
"whole population"[a] $HKAF_{95}^{th}$	78	63	86	66
"whole population"[a] $HKAF_{99}^{th}$	92	85	96	86
Default 3.16 factor	100	100	98	100
CANADIAN POPULATION				
Greatest[b] "distinct subpopulation" $HKAF_{95}^{th}$	> 99	> 99	> 99	> 99
Greatest[b] "distinct subpopulation" $HKAF_{99}^{th}$	> 99	> 99	> 99	> 99
Default 3.16 factor	100	100	> 99	100

a) Based on the median value in the whole Canadian population. b) Based on the median value in adults in Table 6-V.

Symbols: CAss, blood concentration of parent compound (µg/L); RAM, rate of metabolism (µg/h-L of liver).

Intégration et synthèse

7 Discussion générale et conclusions

7.1 Discussion générale

L'objectif de cette thèse consistait à caractériser la composante toxicocinétique du facteur d'ajustement interindividuel (FACH) en fonction de diverses considérations qui faisaient chacune l'objet d'un article spécifique (voir sections 1.5 et 1.6). Cette caractérisation a permis d'évaluer la justesse de la valeur de 3,16 attribuée par défaut à cette composante (Dourson *et al.*, 1996; Renwick et Lazarus, 1998), pour les diverses conditions de modélisation étudiées. L'objet de la présente thèse étant l'étude de la variabilité toxicocinétique interindividuelle, il était nécessaire de faire appel à la modélisation TCBP plutôt qu'à la modélisation à compartiments classique. En effet, l'approche fondamentale de cette étude consistait à comparer les résultats de modélisation simulés pour des adultes à ceux simulés chez d'autres groupes de la population. De tels résultats n'auraient pu être obtenus en grand nombre par modélisation à compartiments classique. La raison en est que ces modèles se basent sur les données expérimentales pour être construits (Gibaldi et Perrier, 1982; Krishnan et Andersen, 2008). Or, en raison de considérations éthiques, de telles données sont difficilement disponibles chez les sous-groupes présumés sensibles de la population (c.-à-d. nouveau-nés, enfants, femmes enceintes, aînés, etc.). En se basant sur les caractéristiques physiologiques des individus plutôt que sur des mesures expérimentales, la modélisation TCBP permet de contourner cette difficulté. Pour les mêmes raisons, les approches bayésiennes de modélisation (voir chapitre 1) n'ont pas été retenues.

Ceci étant établi, il importait de choisir une approche TCBP se prêtant bien à la simulation probabiliste des doses internes qui soit cohérente avec les différences en fonction de l'âge des relations entre paramètres physiologiques et données morphologiques telles que le poids et la taille. Ces différences sont bien documentées (Clewell *et al.*, 2002; 2004; Faustman et Ribeiro, 1990; Krishnan et Johanson, 2005; Haddad *et al.*, 2001; Price *et al.*, 2003a; Valcke et Krishnan, 2009), et elles n'auraient pu être prises en compte si la méthode traditionnelle utilisée pour définir les débits et volumes des divers compartiments des

modèles TCBP avait été suivie pour chaque sous-groupe investigué. En effet, cette méthode, destinée à la modélisation pour un individu adulte moyen, consiste à attribuer un pourcentage constant du poids corporel et du débit cardiaque, lui-même calculé à partir d'une constante (ex. : 15 L/h-kg$^{0.75}$), pour évaluer respectivement le volume et le débit de chaque compartiment TCBP (Krishnan et Andersen, 2008). Nong *et al.* (2006) ont beau avoir utilisé des distributions variables pour définir les paramètres physiologiques de modèles TCBP probabilistes de divers groupes d'âge, ces paramètres n'étaient pas corrélés au poids corporel, ni entre eux. Le recours à des équations spécifiques à l'âge et/ou au sexe, pour calculer ces paramètres à partir de l'âge et des valeurs corrélées de poids et de taille (ex. : Beaudoin *et al.*, 2010; Mörk et Johanson, 2010; Clewell *et al.*, 2004; Price *et al.*, 2003a; 2003b; Pelekis *et al.*, 2003), permet de corriger cette lacune et de s'assurer que les valeurs de paramètres physiologiques retenues à chaque simulation de Monte-Carlo soient cohérentes entre elles. Cette cohérence est importante puisqu'autrement, des combinaisons de valeurs, irréalistes du point de vue de la biologie, pourraient se produire (par exemple, le 50^{e} centile du poids corporel combiné au 10^{e} centile du volume et au 95^{e} centile du débit hépatique), entraînant une surestimation de la variabilité toxicocinétique.

Ultimement, les équations de Price *et al.*, (2003b) ont été retenues, principalement par souci de cohérence avec la base de données P^{3}M, utilisant ces équations et à laquelle on s'est référé pour obtenir les distributions de poids corporel et de taille issues des données de NHANES. Par souci de constance tout au long de la thèse, les équations publiées par d'autres auteurs en cours de réalisation n'ont pas été considérées. Un « terme de variabilité » a été ajouté comme multiplicateur des équations appliquées à certains paramètres sensibles des modèles. Ces paramètres sont définis dans les articles comme « *variability terms* » et ont été décrits en détail dans l'article I. Aux fins de l'étude de la variabilité toxicocinétique interindividuelle, cet ajout apparaît pertinent étant donné qu'il est logique de croire que des individus de même sexe, âge, poids corporel et taille puissent présenter des variations pour un paramètre physiologique donné (ex. : Thomas *et al.*, 1996). Ces « termes de variabilités » constituent, avec la modélisation de la clairance rénale à

partir des valeurs de filtration glomérulaire et de débit sanguin rénal, une contribution méthodologique originale de la présente thèse en ce qui a trait à la modélisation TCBP. De manière générale, les modèles et algorithmes utilisés ont été validés avec des données expérimentales, mais une incertitude demeure puisque ces données ne concernaient que les adultes (articles I à III), ou encore étaient issues d'essais faits avec des médicaments (article III).

Dans les articles I, II et V, des critères communs relatifs aux différences de valeurs de ratio d'extraction hépatique (E, élevé *vs* faible) et de coefficient de partage sang:air (Pb, élevé *vs* faible) ont guidé le choix des substances retenues. De plus, des critères d'inclusion et d'exclusions spécifiques à chaque étude expliquent que les substances choisies variaient d'un article à l'autre. Notons que dans l'article II, le chloroforme, retenu dans l'article I, a été remplacé par le benzène comme substance avec E élevé et Pb faible car la structure du modèle TCPB du benzène était identique aux autres substances retenues, alors que le modèle du chloroforme incluait un compartiment rénal, absent pour les autres substances. Ainsi, on s'assurait que les différences obtenues dans les résultats pour chaque substance soient bien issues des propriétés des substances, et non de structures différentes dans les modèles utilisés. En effet, des différences dans le nombre de compartiments auraient pu influencer les résultats en affectant la distribution des substances, surtout considérant la courte durée des expositions modélisées. Cet impact du volume de distribution sur les FACHs obtenus pour diverses durées a d'ailleurs été suggéré dans les articles I et II.

Des dépassements de la valeur par défaut de 3,16 ont été observés dans cette thèse uniquement lorsque l'analyse se basait sur la substance-mère. De plus, ces dépassements n'étaient observés presqu'exclusivement lorsqu'on considérait les jeunes enfants, soit les nouveau-nés de moins de 30 jours (articles I à V), et les nourrissons âgés de 1 à 12 mois (articles IV et V). Ainsi, l'exposition au bromoforme par toutes les voies (3,6–7,4), de même qu'au chloroforme par voie orale (4,9) a généré de telles valeurs de FACH dans

l'article I. Dans l'article II, de tels dépassements étaient observés pour l'exposition de 8 ou 24 heures à de faibles niveaux de styrène (3,4), pour des expositions élevées durant 8 heures (3,4 et 5,2), de même que pour l'exposition durant 24 heures (3,4) ou à l'équilibre (6,8) au 1,4-dioxane. Cette valeur pour le 1,4-dioxane a d'ailleurs été reproduite dans l'article V, dans lequel des FACHs > 3,16 ont aussi été obtenus (max. : 3,8) lorsque le 99^{e} centile de la distribution de la concentration sanguine chez les nourrissons (et la femme enceinte) était considéré. L'article III a fait état de dépassements de la valeur par défaut pour les substances présentant un Pb ≥ 100 lors d'une exposition systémique à un substrat du CYP1A2 présentant un E variant entre 0,1 et 0,8 (3,2–6,1), de même que pour une exposition par inhalation aux substrats du CYP2E1, du CYP3A4 et de l'ADH avec un E ≤ 0,8 (3,2–5,4). Dans les cas des substrats du CYP1A2, l'exposition par inhalation pouvait entraîner des FACHs atteignant 12,3 pour des Pb ≥ 50 avec un E ≤ 0,9. Finalement, la valeur par défaut était souvent dépassée pour l'exposition par ingestion pour des substrats présentant des caractéristiques d'E et de Pb variées (Article IV). Ce dépassement pouvait atteindre 6,3 et 28,3 pour les substrats du CYP2E1 et du CYP1A2 respectivement, et était également observé à l'occasion chez les nourrissons dans le cas du CYP1A2, avec une valeur maximale de 4,4.

Outre l'identification des cas où la valeur par défaut était dépassée, cette thèse a mis en lumière les déterminants du FACH. L'article I a notamment démontré que le FACH basé sur la substance mère variait de manière importante en fonction de la voie d'exposition. Par exemple, le FACH le plus élevé obtenu pour une exposition orale au bromoforme était de 7,4 alors que pour l'inhalation et le contact cutané, les valeurs étaient respectivement de 3,6 et 4,4. L'article II a quant à lui démontré une dépendance en fonction de la durée (et de l'intensité) de l'exposition. En particulier, le FACH chez les nouveau-nés, basé sur la Cmax, variait entre 1,4 et 5,2 pour le styrène et entre 2,2 et 4 pour le 1,4-dioxane pour des expositions de 24 heures ou moins. Par contre, en considérant les conditions à l'équilibre, le FACH était de 2,5 pour le styrène et de 6,8 pour le 1,4-dioxane. Appliqués au contexte de la détermination de la RfD ou de la RfC, les résultats des deux premiers articles faisaient

donc ressortir la nécessité de considérer les conditions à l'équilibre d'une part, séparément pour l'exposition orale et l'exposition par inhalation d'autre part. C'est ce qui a été fait dans les articles III et IV, lesquels ont eu recours à un algorithme toxicocinétique à l'équilibre pour des raisons de simplification. En effet, Pelekis *et al.* (1997) ont démontré que pour des niveaux d'exposition faibles équivalents aux niveaux environnementaux, une telle approche génèrait des résultats très comparables à ceux fournis par les modèles TCBP complets pour l'inhalation tout en requérant beaucoup moins de paramètres. L'article IV a mis en évidence que cela s'appliquait aussi à l'exposition par ingestion.

Les articles III et IV ont notamment mis en évidence que la valeur du FACH dépendait des propriétés physico-biochimiques des substances (E, Pb, ainsi que la voie métabolique). De plus, les FACHs obtenus sur la base de la concentration sanguine en substance mère étaient généralement plus élevés pour l'exposition par inhalation que pour l'exposition systémique en mg/kg-d (article III), et étaient encore plus élevés pour l'exposition par ingestion (article IV). Ainsi, la variation adulte/enfant de la biodisponibilité orale découlant du phénomène de premier passage hépatique, lequel fut clairement mis en évidence expérimentalement pour le TCE et le chloroforme par Weisel et Jo (1996), a résulté en ce que pour une même dose ingérée par unité de poids corporel, la dose de substance mère atteignant la circulation systémique après son premier passage au foie pouvait être significativement plus élevée chez les jeunes enfants que chez les adultes (Tableau 5-II de l'article IV). Ceci était surtout vrai pour les substances moyennement à fortement métabolisées (c.-à.-d présentant un E variant entre ≈ 0,5 et 0,99) et pour presque tous les substrats du CYP1A2 en raison de l'immaturité particulièrement importante de ce système enzymatique chez les jeunes enfants. Ainsi, la Figure 5.3 a montré que ne pas considérer le phénomène de premier passage hépatique pouvait résulter en une sous-estimation importante (par un facteur allant jusqu'à environ 10) des FACHs basés sur la concentration sanguine de substances mères ingérées. Comme cette omission revient à assumer une biodisponibilité constante de 100 % de ces substances, elle équivaut à négliger la variabilité interindividuelle de ce qu'on pourrait appeler le « taux de contact systémique » de celles-ci. Pour l'inhalation, cela

reviendrait à négliger la variabilité interindividuelle du taux de ventilation alvéolaire. Cette négligence constitue d'ailleurs en partie la base du raisonnement de Ginsberg *et al.* (2010) qui suggèrent que l'application du même facteur d'incertitude interindividuel pour la détermination, par la U.S. EPA, de la RfC et de la RfD omet de protéger les jeunes enfants dans le premier cas.

Ultimement donc, les matrices obtenues aux articles III et IV font ressortir l'importance de prendre en compte à la fois la variabilité de la biodisponibilité et de la clairance systémique pour évaluer les FACHs, et non pas seulement de la seconde tel que suggérée par l'IPCS (2005) pour l'exposition orale et retenue pour élaborer la figure 4.3. Toutefois, Brochu *et al.* (2010) ont fait état d'un facteur de variabilité populationnelle interindividuelle d'environ 11 basé sur le rapport entre le 99^{e} et le 1er centile des taux de ventilation alvéolaire ajustés au poids corporel, qui sont des taux de contact. Ce rapport est fort différent des rapports équivalents pouvant être tirés du Tableau 6-IV de l'article V considérant les nouveau-nés et l'adulte respectivement, pour la concentration sanguine de 1,4-dioxane (22) et de benzène (2). Pour éviter des erreurs appréciables sur l'estimation de la variabilité des doses internes et donc des FACH, on constate ainsi qu'il est important d'utiliser la modélisation toxicocinétique qui considère à la fois le taux de contact et la cinétique interne.

L'aspect le plus intéressant de l'article V est toutefois qu'il permette de faire le lien entre les concepts scientifiques sous-tendant la variabilité populationnelle et les considérations pratiques chères au gestionnaire de risque. Par exemple, le fait que l'impact de la composition de la population sur les FACHs soit apparu marginal lui permet de ne pas se préoccuper de la démographie de la population qu'il couvre. Cet aspect est intéressant quant au potentiel de généralisation, peu importe la population, de résultats pertinents à l'évaluation de la variabilité toxicocinétique populationnelle et issue d'une étude précise. On peut penser aux campagnes de surveillance biologique à grande échelle, comme NHANES. Le gestionnaire de risque n'aurait pas non plus, selon les résultats obtenus, à se préoccuper

du choix du référent pour calculer le FACH, soit l'adulte médian ou l'individu médian d'une population. Cependant, l'article V a démontré que les fractions de chaque sous-groupe couvertes par le FACH ne sont pas les mêmes selon l'approche utilisée. Ceci constitue une question fondamentale à laquelle il importe de s'arrêter (Hattis *et al.*, 1999b).

Ainsi, le calcul du FACH par l'approche « population entière » (*whole population* dans l'article) résulte en un facteur, ici toujours < 3,16, qui couvrira une proportion très importante de la population globale, mais qui pourrait omettre une proportion significative des individus appartenant à un sous-groupe de la population qui soit particulièrement sensible du point de vue de la toxicocinétique, mais comprenant peu d'individus. En utilisant l'approche « sous-groupe distinct » (*distinct subpopulation* dans l'article), qui requiert ultimement de retenir le FACH le plus élevé parmi ceux déterminés pour chaque sous-groupe, même le sous-groupe le plus sensible sera protégé adéquatement. En revanche, le gain à l'échelle de la couverture de l'ensemble de la population serait minimal, et ce, malgré une valeur de FACH plus élevée, donc plus prudente. L'article V a aussi fait ressortir que la différence résultant de la considération du 99^{e} centile de la dose interne plutôt que du 95^{e} était comparable entre les approches utilisées pour déterminer le FACH, mais pas entre les substances considérées. Ainsi, cette différence, pour la substance mère, était plus importante pour le 1,4-dioxane (écart d'environ 25–30 %) que pour le benzène (moins de 10 %), ce qui illustre que la variabilité des doses internes est spécifiques aux substances. Un tel écart peut être significatif du point de vue normatif lorsqu'il résulte en des FACHs se situant de chaque côté de la valeur par défaut de 3,16. Le Tableau 7-I fait état de cas, impliquant toujours des FACHs calculés selon l'approche « sous-groupe distinct », où cela a été observé dans les divers articles de cette thèse. Pour une situation donnée, la recherche de l'équilibre entre la nécessité de protéger un sous-groupe identifié comme sensible d'une part, et la prise en compte de la fraction de la population générale que ce sous-groupe représente d'autre part, orientera le choix de l'approche et du centile retenu par le gestionnaire de risque.

Tableau 7-I : Cas où le FACH est passé au-delà de la valeur de 3,16 lorsque le 99^{e} centile de la dose interne mesurée était considéré plutôt que le 95^{e} centile.

Article	Sous-groupe	Exposition	Entité toxique ou propriétés de la substance mère	FACH selon : 95^{e}; 99^{e} centile
I	Nouveau-nés	Cutanée	TCE	3,1; 3,5
			TCA, (métabolite du PCE)	2,9; 3,9
II	Nouveau-nés	Inhalation à l'équilibre	Styrène	2,5; 3,2
		Inhalation à la RfC, 8 heures	1,4-dioxane	3,1; 3,4
III	Nouveau-nés	Orale, substrats du CYP2E1	E = 0,3; Pb = 3000	2,7; 4,0
		Orale, substrats du CYP1A2	E = 0,1, Pb = 3000	3,1; 3,6
		Inhalation, substrats du CYP2E1	E = 0,2, Pb = 100	3,1; 3,5
			E = 0,5, Pb = 50	3,1; 3,6
		Inhalation, substrats du CYP1A2	E = 0,8, Pb = 20	3,3; 3,1
		Inhalation, substrats du CYP3A4	E = 0,2, Pb = 100	2,9; 3,2
			E = 0,7, Pb = 100	2,9; 3,4
		Inhalation, substrats de l'ADH	E = 0,8, Pb = 300	2,9; 3,8
IV	Nouveau-nés	Ingestion, substrats du CYP2E1	E = 0,2, Pb = 3000	3,1; 4,3
			E = 0,2, Pb = 10000	3,1; 4,1
			E = 0,3, Pb = 300	3,1; 3,8
			E = 0,7, Pb = 20	3,1; 3,9
V	Nourrissons	Inhalation	1,4-dioxane	3,0; 3,8
	Femmes enceintes		1,4-dioxane	2,7; 3,5

Abréviations : ADH, alcool déshydrogénase, CYP, cytochrome p-450; E, ratio d'extraction hépatique, RfC, concentration de référence; PCE/TCE, per-/tri-chloroéthylène; TCA, acide trichloroacétique.

Cette dernière question met en lumière une limite importante associée à la présente thèse, soit la détermination des bornes d'âges définissant les divers sous-groupes considérés. Ces bornes ont un impact direct sur les résultats obtenus. Ainsi, si l'on avait défini les nouveau-nés comme étant âgés de 90 jours ou moins plutôt que de 30 jours ou moins, cela aurait potentiellement entraîné la détermination de distributions fort différentes pour définir le poids corporel bien sûr, mais surtout les divers contenus hépatiques en enzymes de biotransformation considérées. En effet, le développement de ces enzymes peut être rapide dans les premiers mois de vie, et il varie d'une enzyme à l'autre (Valcke et Krishnan, 2009). Donc, considérer plus d'individus plus âgés au sein des nouveau-nés, et moins

d'individus plus jeunes au sein des nourrissons (âgés de 1-12 mois dans les articles IV et V) aurait eu pour effet potentiel de diminuer la sensibilité de ces deux sous-groupes découlant de l'immaturité de ces enzymes. Ceci aurait vraisemblablement généré des FACHs déterminés à partir de la mesure de substances mères plus faibles que ceux ayant été calculés, les faisant possiblement passer dans certains cas de > 3,16 comme indiqué dans les articles, à < 3,16.

Il importe de préciser que l'âge attribué à chaque individu d'un sous-groupe donné lors des simulations de Monte-Carlo était constant et correspondait à l'âge médian de ce sous-groupe. En effet, l'analyse des résultats fournis par la base de données P3M montre que chez les non-adultes, l'âge est fortement corrélé avec le poids et la taille, et cette corrélation varie selon le sexe. Lors des simulations de Monte Carlo, des corrélations multiples auraient donc dû être introduites si la variation de l'âge avait été considérée. Ceci aurait compliqué l'interprétation des résultats sans pour autant résulter nécessairement en un gain de robustesse. En effet, l'âge n'était requis que pour calculer le volume de tissus adipeux (donc le débit sanguin correspondant) chez les 1-18 ans, le volume des os chez les 0-4 ans ainsi que le débit sanguin aux tissus richement perfusés dans tous les sous-groupes. À l'exception du volume de tissus adipeux, ces paramètres étaient peu sensibles dans les modèles TCBP et n'étaient requis que dans les articles I et II. De plus, les étendues d'âges chez les sous-groupes les plus sensibles étaient faibles (entre 1 mois et 2 ans, chez les moins de 4 ans), ce qui réduit la possible erreur découlant du choix d'une valeur unique et médiane d'âge. Finalement, les étendues d'âges ont été considérées lors de l'interrogation de la base de données P3M pour obtenir les distributions de poids corporel et de taille de chaque sous-groupe, ce qui permet de tenir compte indirectement de la variabilité de l'âge. Pour toutes ces raisons, l'impact de l'attribution de l'âge médian du sous-groupe auquel il appartient à chaque individu modélisé apparaît minime. Qui plus est, la corrélation de l'âge avec le poids corporel et la taille chez les non-adultes aurait résulté en une diminution de la variabilité des doses internes; les résultats obtenus vont donc dans le sens d'une évaluation plus conservatrice (élevée) des FACHs obtenus.

Comme autre limite, on peut relever le fait que l'impact du polymorphisme des enzymes hépatiques sur la variabilité toxicocinétique populationnelle n'a pas été évalué. En effet les substances investiguées sont des substrats du CYP2E1 pour lequel le polymorphisme ne contribue que peu à la variabilité de l'expression de l'enzyme à l'échelle populationnelle (Neafsey *et al.*, 2009). Par contre, l'impact du polymorphisme à l'échelle populationnelle a été bien documenté pour la glutathione-s-transférase (GST), une enzyme qui métabolise certains contaminants environnementaux comme le dichlorométhane et l'acide dichloroacétique (Blackburn *et al.*, 2000; El-Masri *et al.*, 1999; Jonsson et Johanson, 2001). L'analyse de cette question aurait cependant requis de définir un sous-groupe populationnel supplémentaire. Combiné aux limites d'âge, ceci se serait soldé en certains groupes au nombre très restreint d'individus, ce qui aurait rendu difficile, voire impossible, l'analyse des résultats en découlant en raison d'une représentativité très réduite. Notons de plus que le polymorphisme de la GST peut en fait, et dépendamment de l'entité causant l'effet toxique non cancérigène (substance mère ou métabolite), résulter en une diminution de la sensibilité des individus touchés en comparaison de ceux déjà investigués dans la présente thèse (Jonsson et Johanson, 2001; Blackburn *et al.*, 2000). Leur considération apparaissait donc moins pertinente.

La variabilité populationnelle du contenu hépatique en enzymes de biotransformation et en protéines microsomales constitue enfin une source d'incertitude qu'il importe de relever. En effet, la méthodologie suivie implique l'ajustement de la clairance intrinsèque en fonction de ces variables. Les données sur ce contenu sont basées sur un nombre relativement restreint d'individus analysés (Lipscomb et Poet, 2008; Johnsrud *et al.*, 2003; Lipscomb *et al.*, 2003; Sonnier et Cresteil, 1998; Lacroix *et al.*, 1997; Shimada *et al.*, 1994). La représentativité de ceux-ci pour l'ensemble de la population est donc incertaine. Également, malgré que les CYP puissent être retrouvées ailleurs que dans le foie (Ronis *et al.*, 1996), il a été assumé tout au long de cette thèse que le métabolisme n'avait lieu dans aucun autre organe, à l'exception des reins dans le cas du chloroforme et du PCE (article I,

Corley *et al.*, 1993; Gearhart *et al.*, 1993). Cette omission n'aurait toutefois pas d'impact important sur les résultats de modélisations toxicocinétiques (Yoon *et al.*, 2007), ce qui a également été observé dans les présents travaux. Évidemment, tout exercice de modélisation TCBP impose de faire des choix dans les paramètres physiologiques retenus afin d'obtenir un juste équilibre entre robustesse et complexité du modèle. On ne peut non plus considérer des paramètres pour lesquels les données existantes ne sont pas suffisantes pour permettre leur usage dans les modèles, même si on suspecte qu'ils pourraient être importants (ex. : les protéines de transport de la paroi intestinale mentionnées dans l'article IV). Ces limites sont inhérentes à toute approche de modélisation.

Des analyses de sensibilité comme celles réalisées dans l'article III peuvent contribuer à l'identification des déterminants de la sensibilité toxicocinétique pour des substances présentant des propriétés physico-chimiques données. Lorsqu'elles sont réalisées en fonction de la voie d'exposition comme à la Figure 7.1, de telles analyses permettent de focaliser l'analyse en fonction de la VTR (RfD ou RfC) pour laquelle un FACH est défini. Ainsi, cette figure montre que la clairance intrinsèque (Clint) consiste en un paramètre influant sur la SSC du chloroforme fortement métabolisé (A, C), mais pas du PCE faiblement métabolisé. De même, la clairance intrinsèque du chloroforme a une plus forte influence sur la SSC suite à une exposition par voie orale (C) que par inhalation (A) ce qui confirme l'effet de premier passage hépatique mentionné plus haut. Également, la ventilation alvéolaire (Qp), qui détermine la clairance pulmonaire, est un paramètre plus sensible pour le PCE, faiblement métabolisé, que pour le chloroforme. Cette observation explique que le FACH des nouveau-nés obtenu dans l'article I pour le chloroforme était plus élevés pour l'exposition orale constante en mg/kg-d que pour l'exposition par inhalation, et que ce n'était pas le cas pour le PCE. Le volume de tissus adipeux (Vf) présente également des indices de sensibilité plus élevés pour le PCE que pour le chloroforme, ce qui est cohérent avec sa liposolubilité plus grande et l'impact suggéré du volume de distribution sur la cinétique de courtes expositions, mentionné dans l'article II.

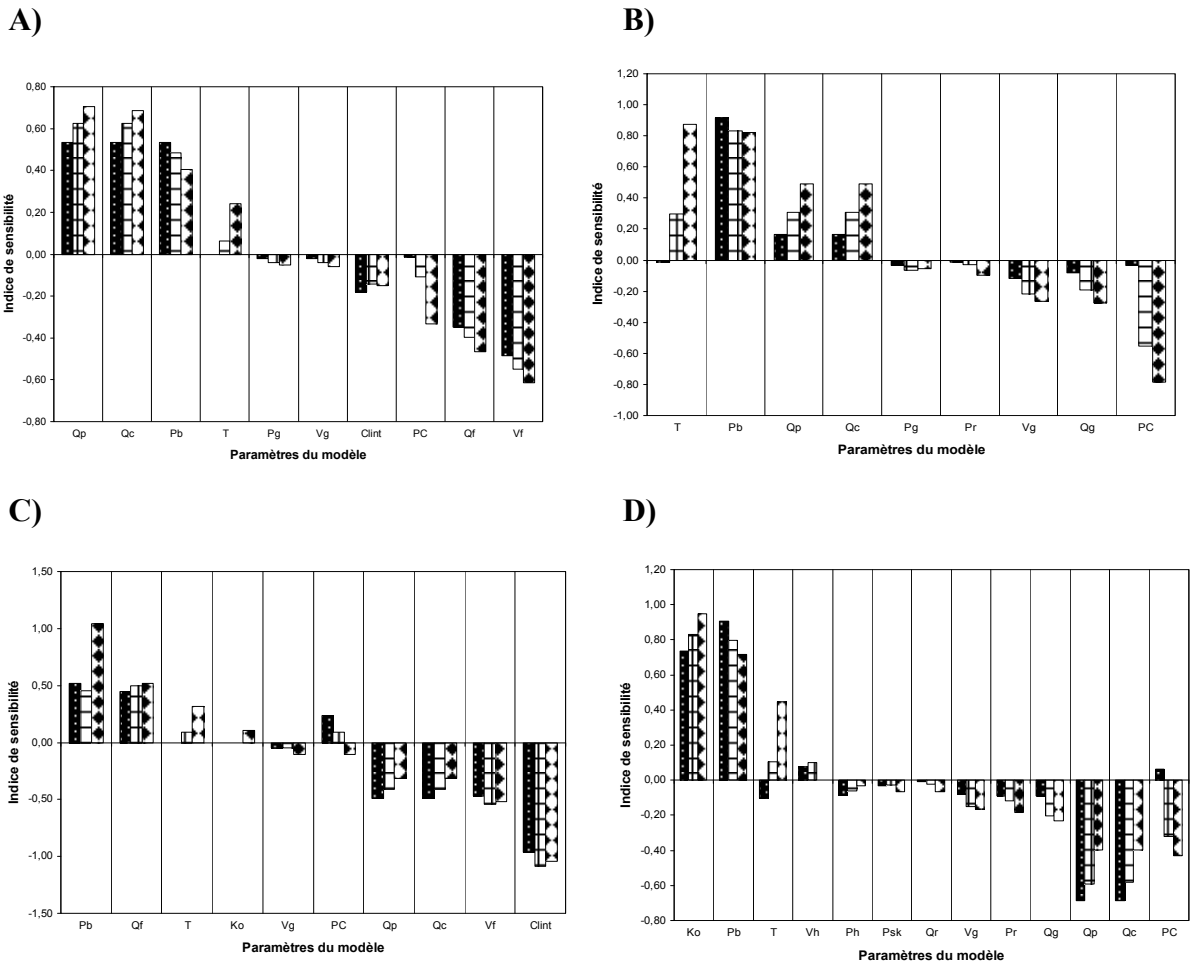


Figure 7.1 : Sensibilité de la surface sous la courbe 24 h pour le chloroforme (A, C) et le PCE (B, D) envers les paramètres des modèles TCBP décrits dans l'article I pour les nouveau-nés, les enfants et les adultes (de gauche à droite). Les indices de sensibilité ont été calculés comme dans l'article III pour les scénarios d'exposition décrits dans l'article I pour l'inhalation (A, B) et la voie orale (C, D): Clint, Clairance intrinsèque; Ko, constante d'absorption; PC, poids corporel; Px, coefficient de partage tissu:sang (f, foie; g, tissus adipeux; h, hautement perfusés; r, reste du corps) et sang:air (b); Qp, taux de ventilation alvéolaire, débit cardiaque; Qx, débit et Vx, volume tissulaire; T, taille.

Les analyses de sensibilité réalisées dans cette thèse doivent cependant être interprétées avec prudence, car les paramètres concernés sont corrélés entre eux. Ainsi, un changement de la SSC observé en modifiant le débit hépatique de 10 % peut être causé autant par le changement du débit hépatique que par celui du débit des tissus richement perfusés, lequel constitue le « tampon » du débit cardiaque total afin de maintenir le bilan de masse. Il en va de même pour tout changement dans le poids corporel, à partir duquel plusieurs valeurs de paramètres sont calculées. En prenant en compte adéquatement ces corrélations toutefois (ex. : Fenneteau *et al.*, 2009), de telles analyses permettent de mettre en lumière les paramètres physiologiques à prioriser quand l'on veut orienter la recherche de données populationnelles afin de diminuer l'incertitude sur la valeur du FACH. En effet, de l'incertitude sur un paramètre d'entrée d'un modèle physiologique peut entraîner de l'incertitude sur la distribution de la dose interne modélisée par simulations de Monte-Carlo (U.S. EPA, 2001). Comme le montre le Tableau 7-II, la variation pouvant découler de cette incertitude sur les centiles pertinents à l'élaboration du FACH peut être assez importante (souvent autour de 10 % et jusqu'à 24 %), ce qui peut modifier le jugement porté sur l'adéquation de la valeur par défaut de 3,16. Dans ce contexte, des analyses d'incertitude par simulations de Monte-Carlo à deux dimensions (ex. : Ragas *et al.*, 2009, Simon, 1999), ainsi que des analyses de variabilité (ex.; Sweeney *et al.*, 2003) sont deux approches pouvant être mises à profit.

Tableau 7-II : Impact, en pourcentage de changement, de modifications dans la distribution définissant les trois paramètres les plus influents sur la concentration sanguine pour les scénarios de clairance C, E et F de l'article III[a)], sur les centiles pertinents à l'élaboration des FACHs chez les nouveau-nés.

	Modification et descripteurs statistiques considérés								
	L'écart-type est réduit de 20 %			La distribution devient triangulaire			Inversion des formes lognormales ⇔ normales		
Scénario Paramètre	Ad., 50^{e}	Ad., 95^{e}	N.-né,95^{e}	Ad., 50^{e}	Ad., 95^{e}	N.-né, 95^{e}	Ad., 50^{e}	Ad., 95^{e}	N.-né, 95^{e}
C									
Vl*	-0,6 %	-1,3 %	2,1 %	-0,2 %	-0,2 %	1,1 %	0,2 %	0,4 %	1,9 %
Qp*	-0,2 %	-0,4 %	0,6 %	-0,8 %	-0,7 %	1,3 %	-0,5 %	0,4 %	2,7 %
ENZ	-3,8 %	-6,4 %	-2,4 %	-8,9 %	-6,2 %	-10,7 %	-6,0 %	1,1 %	-1,8 %
E									
Vl*	-1,2 %	-3,1 %	-0,9 %	-0,2 %	-1,7 %	-0,2 %	2,4 %	-1,0 %	3,0 %
ENZ	-2,7 %	-9,9 %	-8,9 %	-11,7 %	-9,1 %	-24,2 %	-7,1 %	3,0 %	2,9 %
FG	0,0 %	-1,1 %	-5,6 %	-1,1 %	-1,7 %	-12,1 %	0,2 %	-0,5 %	1,9 %
F									
FG	18,4 %	15,6 %	-12,7 %	-1,4 %	2,0 %	-13,1 %	0,0 %	0,5 %	15,9 %
PC	0,1 %	-0,8 %	0,7 %	6,1 %	9,2 %	7,7 %	0,4 %	0,1 %	4,2 %
T	-0,5 %	0,1 %	2,2 %	-0,2 %	0,2 %	-1,2 %	-0,1 %	1,0 %	-1,7 %

a) Voir Figure 4.6, article III. Ces scénarios sont ceux pour lesquels les pourcentages de changement les plus élevés pnt été observés.

* : Comme ces variables sont calculées à partir du poids corporel, les résultats obtenus découlent des modifications aux distributions qui correspondent à leur « terme de variabilité » respectif (voir article 3).

Abréviations : Ad, adultes; ENZ, contenu en enzymes hépatiques de biotransformation; FG, filtration glomérulaire, N-Né, nouveau-nés; PC, poids corporel; Qp, taux de ventilation alvéolaire; T, taille; Vl, volume hépatique

Une fois les déterminants de la sensibilité toxicocinétique identifiés, il devient aisé d'expliquer l'ampleur du FACH pour chaque sous-groupe, à considérations données. Ainsi, les FACHs les plus élevés basés sur la substance mère étaient obtenus chez de jeunes enfants en raison de leurs taux de contact plus élevés par unité de poids corporel et de leur

clairance hépatique diminuée comparée à chez l'adulte. Pour l'inhalation et l'exposition systémique, ces FACHs étaient également plus élevés lorsque E était tel que la clairance hépatique était enzyme-dépendante et contribuait à une part importante de la clairance totale (voir Figures 4.3, 4.5 et 4.7). Pour l'ingestion, c'était de plus le cas lorsque la valeur de E, et donc l'effet de premier passage hépatique, était élevée (Figure 5.2). Tout autre paramètre étant égal, le FACH chez les nouveau-nés croissait avec le Pb en raison de l'incapacité qui en résulte pour les jeunes enfants de « profiter » de leur ventilation alvéolaire, plus élevée par unité de poids corporel que les adultes (Valcke et Krishnan, 2009), pour baisser leurs niveaux sanguins de substance mère. En ce qui a trait aux FACHs basés sur la production de métabolites, les plus élevés étaient observés chez le sous-groupe présentant à la fois une dose interne de substance mère élevée et une capacité métabolique importante. Ces conditions étaient le plus souvent observées chez la femme enceinte en raison de son taux de contact élevé combiné à un débit hépatique important, mais aussi à l'occasion chez les enfants plus âgés (article I et II), les aînés (articles I et III), de même que les nouveau-nés quand leur taux de contact était suffisamment élevé pour que la quantité de substance mère dans le sang puisse compenser leur métabolisme réduit (article I). Toutefois, le FACH dans ces cas n'a jamais dépassé la valeur de 3,16. Cette différence marquée en fonction de l'entité toxique considérée s'explique fondamentalement par l'effet opposé du métabolisme sur la sensibilité toxicocinétique associée à chaque mesure de la dose interne. En effet, un métabolisme faible favorise des concentrations élevées de substance mère dans le sang, mais peu de formation de métabolites; inversement, un métabolisme élevé favorise l'abaissement des niveaux sanguins de substance mère pouvant être métabolisée. Quand ce sont les métabolites qui constituent l'entité toxique générant l'effet sur lequel on peut s'appuyer lors de la détermination des valeurs toxicologiques de référence (ex. : Clewell et Andersen, 2004), de tels résultats sont rassurants. Ainsi, pour une substance donnée, les résultats obtenus dans cette thèse doivent être examinés à la lumière des recommandations de l'IPCS (2005) quant à la nécessité de prendre en considération le mode d'action des substances dans la détermination des FASC. Mais au-delà de l'aspect strictement réglementaire, cette thèse apporte une contribution pertinente de nature plus

fondamentale à l'étude de la physiologie humaine : elle a permis de caractériser « la variabilité de la variabilité » toxicocinétique selon les hypothèses étudiées.

Les travaux décrits ont été réalisés dans le contexte de l'évaluation du risque toxicologique non cancérigène. Toutefois, la tendance actuelle est à l'unification des approches pour l'évaluation des risques pour les substances cancérigènes et non cancérigènes, notamment par l'approche des marges d'exposition (U.S. EPA, 2005; Clewell et Andersen, 2004). La détermination de FACH telle qu'illustrée pourrait s'avérer pertinente dans ce contexte afin de déterminer la contribution à cette marge dont il faut tenir compte pour englober la variabilité toxicocinétique populationnelle. Bien que ce fut brièvement abordé dans l'article I, des recherches supplémentaires devraient aussi porter sur les déterminants du FACH en fonction de la cinétique des métabolites sous diverses formes (stables, réactifs). L'impact, sur la valeur du FACH, de la variabilité interindividuelle du contenu en protéines plasmatique, évoqué dans l'introduction et dans l'article III, constituerait également une avenue de recherche intéressante. Enfin, l'ampleur de la variabilité interindividuelle des doses internes lors d'expositions à des mélanges, avec ses implications sur la détermination du FACH, devrait être étudiée puisque l'élaboration des VTR ne tient pas compte de telles expositions, qui sont la règle plus que l'exception pour les contaminants environnementaux.

7.2 Conclusion

En conclusion, cette thèse a contribué à l'avancement des connaissances dans le domaine de la variabilité toxicocinétique populationnelle. En effet, cette thèse a caractérisé, pour la première fois, le FACH de manière systématique. Cette caractérisation a fait ressortir l'importance de prendre en compte à la fois les conditions d'exposition, les propriétés physico-biochimiques et l'entité toxique des substances, les sous-groupes concernés et la fraction de la population qu'on vise à couvrir en déterminant le FACH. Les cas où la valeur par défaut de 3,16 était dépassée ont donc pu être mis en évidence. Ces cas consistaient toujours en des FACHs basés sur des substances mères exhibant des combinaisons

variables de propriétés physico/biochimiques, dépendamment du scénario d'exposition considéré, et ne concernaient que les jeunes enfants âgés d'au plus 1 an. Les FACHs les plus élevés (jusqu'à 28,3) ont été obtenus pour l'exposition par ingestion, suivi de l'inhalation (jusqu'à 12,3). La caractérisation, par modélisation physiologique, de la variabilité interindividuelle des doses internes pour les polluants ingérés constitue d'ailleurs une contribution particulièrement originale de cette thèse. En effet, cette question n'avait été que peu étudiée jusqu'à aujourd'hui.

Les travaux menés ont requis le développement d'approches méthodologiques novatrices dans le domaine de la modélisation physiologique. D'abord, une méthode pour la détermination de paramètres physiologiques cohérents pour un individu donné, mais reflétant également la variabilité plausible entre des individus aux mêmes caractéristiques morphologiques a été développée. Ensuite, une méthode pour calculer la clairance rénale sur la base physiologique pour des substances pour lesquelles on ne dispose pas de données expérimentales a été proposée. Finalement, des distributions théoriques de doses internes pour une population ont été « reconstruites » en fonction des caractéristiques démographiques de celle-ci.

La portée de cette thèse concerne les activités des autorités de santé publique en ce qui a trait au domaine de l'analyse du risque toxicologique. En effet, elle contribue à diminuer les incertitudes relatives à la variabilité toxicocinétique interindividuelle et au facteur devant être appliqué pour en tenir compte, ainsi qu'à la caractérisation des sous-groupes de la population les plus sensibles à considérations données. Ainsi, les résultats obtenus peuvent autant être interprétés dans la perspective de l'évaluation de l'adéquation de la valeur par défaut de 3,16 pour protéger les individus sensibles de la population, que dans celle du remplacement de cette valeur par des facteurs « basés sur la science » comme le suggère l'IPCS (2005).

Références bibliographiques

Abraham, K., Mielke, H., Huisinga W., Gundert-Remy, U. 2005a. Elevated internal exposure of children in simulated acute inhalation of volatile organic compounds: effects of concentration and duration. *Arch. Toxicol. 79*, 63-73.

Abraham, K., Mielke, H., Huisinga W., Gundert-Remy, U. 2005b. Internal exposure of children by simulated acute inhalation of volatile organic compounds: the influence of chemical properties on the child/adult concentration ratio. *Basic Clin. Pharmacol Toxicol. 96*, 242-243

Alcorn, J., McNamara, P. J. 2002. Ontogeny of hepatic and renal systemic clearance pathways in infants: part I. *Clin. Pharmacokinet*. *41*, 959-998.

Alcorn, J., McNamara, P. J. 2003. Pharmacokinetics in the newborn. *Adv. Drug Deliv. Rev*. *55*, 667-686.

Arms, A. D., Travis, C. C. 1988 Reference physiological parameters in pharmacokinetic modeling. U.S. Environmental Protection Agency. EPA/600/6-88/004. Washington D.C.

Beaudoin, R., Micallef, S., Brochot, C. 2010. A stochastic whole-body physiologically based pharmacokinetic model to assess the impact of inter-individual variability on tissue dosimetry over the human lifespan. *Regul. Toxicol.Pharmacol*. *57*, 103-116.

Beck, B. D., Mattuck, R. L., Bowers, T. S. 2002. Adult-child differences in the intraspecies uncertainty factor: a case study using lead. *Human Ecol. Risk Assess*. *8*, 877-884.

Blackburn, A. C., Tzeng, H. F., Anders, M. W., Board, P. G. 2000. Discovery of a functional polymorphism in human glutathione transferase zeta by expressed sequence tag database analysis. *Pharmacogenetics. 10*, 49-57.

Bois, F. Y., Jackson, E. T., Pekari, K., Smith, M. T. 1996. Population toxicokinetics of benzene. *Environ Health Perspect*. *104 Suppl 6:1405-11*., 1405-1411.

Bois, F. Y. 2001. Application of population approaches in toxicology. *Toxicol. Lett. 120*, 358-394

Bois, F.Y., 1999. Analysis of PBPK models for risk characterization. *Ann. N. Y. Acad. Sci*. *895*, 317-337.

Bois, F. Y. 2000. Statistical analysis of Clewell *et al.* PBPK model of trichloroethylene kinetics. *Environ. Health Perspect*. *108 Suppl 2:307-16.*, 307-316.

Brochu, P., Brodeur, J., Krishnan, K. 2011. Derivation of physiological inhalation rates in children, adults, and elderly based on nighttime and daytime respiratory parameters. *Inhal. Toxicol. 23*, 74-94.

Brown, E. A., Shelley, M. L., Fisher, J. W. 1998. A pharmacokinetic study of occupational and environmental benzene exposure with regard to gender. *Risk Anal*. *18*, 205-213.

Calabrese, E. J. 1985. Uncertainty factors and interindividual variation. *Regul. Toxicol. Pharmacol*. *5*, 190-196.

Chiu, W. A., Okino, M. S., Evans, M. V. 2009. Characterizing uncertainty and population variability in the toxicokinetics of trichloroethylene and metabolites in mice, rats, and humans using an ubdated database, physiologically based pharmacokinetic (PBPK) model, and bayesian approach. *Toxicol. Appl. Pharmacol*. *241*, 36-60.

Clewell, H. J., Andersen, M. E. 2004. Applying mode-of-action and pharmacokinetic considerations in contemporary cancer risk assessments: an example with trichloroethylene. *Crit. Rev. Toxicol. 34*, 385-445.

Clewell, H. J., Gearhart, J. M., Gentry, P. R., Covington, T. R., VanLandingham, C. B., Crump, K. S., Shipp, A. M. 1999. Evaluation of the uncertainty in an oral reference dose for methylmercury due to interindividual variability in pharmacokinetics. *Risk Anal*. *19*, 547-558.

Clewell, H. J., Teeguarden, J., McDonald, T., Sarangapani, R., Lawrence, G., Covington, T., Gentry, R., Shipp, A. 2002. Review and evaluation of the potential impact of age- and gender-specific pharmacokinetic differences on tissue dosimetry. *Crit. Rev. Toxicol*. *32*, 329-389.

Clewell, H. J., Gentry, P. R., Covington, T. R., Sarangapani, R., Teeguarden, J. G. 2004. Evaluation of the potential impact of age- and gender-specific pharmacokinetic differences on tissue dosimetry. *Toxicol. Sci*. *79*, 381-393.

Clewell, H.J. III., Andersen, M. E. 1996. Use of physiologically based pharmacokinetic modeling to investigate individual versus population risk. *Toxicology*. *111*, 315-329.

Corley, R. A., Mendrala, A. L., Smith, F. A., Staats, D. A., Gargas, M. L., Conolly, R. B., Andersen, M. E., Reitz, R. H. 1990. Development of a physiologically based pharmacokinetic model for chloroform. *Toxicol. Appl. Pharmacol. 103*, 512-527.

Covington, T. R., Gentry, P. R., Van Landingham, C. B., Andersen, M. E., Kester, J. E., Clewell, H.J. 2007. The use of Markov chain Monte Carlo uncertainty analysis to support a Public Health Goal for perchloroethylene. *Regul. Toxicol. Pharmacol*. *47*, 1-18.

Cresteil, T. 1998. Onset of xenobiotic metabolism in children: toxicological implications. *Food Addit. Contam. 15 Suppl*, 45-51.

Crom, W.R. 1994. Pharmacokinetics in the child. *Environ Health Perspect*. *102 Suppl 11*, 111-117.

Cronin, W. J., Oswald, E. J., Shelley, M. L., Fisher, J. W., Flemming, C. D. 1995. A trichloroethylene risk assessment using a Monte Carlo analysis of parameter uncertainty in conjunction with physiologically-based pharmacokinetic modeling. *Risk Anal*. *15*, 555-565.

Dawling, S., Crome, P. 1989. Clinical pharmacokinetic considerations in the elderly. An update. *Clin. Pharmacokinet*. *17*, 236-263.

Delic, J. I., Lilly, P. D., MacDonald, A. J., Loizou, G. D. 2000. The utility of PBPK in the safety assessment of chloroform and carbon tetrachloride. *Regul. Toxicol. Pharmacol*. *32*, 144-155.

DeWoskin, R. S., Thompson, C. M. 2008. Renal clearance parameters for PBPK model analysis of early lifestage differences in the disposition of environmental toxicants. *Regul. Toxicol. Pharmacol. 51*, 66-86.

DeWoskin, R. S., Lipscomb, J. C., Thompson, C., Chiu, W.A., Schlosser, P. M., Smallwood, C., Swartout, J., Teuschler, L. K., Marcus, A. 2007. Pharmacokinetic /Physiologically Based Pharmacokinetic Models in Integrated Risk Information System. DANS: J. C. Lipscomb, E. Ohanian, E. (Eds.), *Toxicokinetics and Risk Assessment*, Informa Healthcare, New York NY, pp. 301-348.

Dorne, J. L., Renwick,A. G. 2005. The refinement of uncertainty/safety factors in risk assessment by the incorporation of data on toxicokinetic variability in humans. *Toxicol. Sci*. *86*, 20-26.

Dorne, J. L., Walton, K., Renwick, A. G. 2005. Human variability in xenobiotic metabolism and pathway-related uncertainty factors for chemical risk assessment: a review. *Food Chem. Toxicol*. *43*, 203-216.

Dourson, M., Charnley, G., Scheuplein,R. 2002. Differential sensitivity of children and adults to chemical toxicity. II. Risk and regulation. *Regul. Toxicol. Pharmacol*. *35*, 448-467.

Dourson, M. L. Stara, J. F. 1983. Regulatory history and experimental support of uncertainty (safety) factors. *Regul. Toxicol. Pharmacol*. *3*, 224-238.

Dourson, M. L., Felter, S. P., Robinson, D. 1996. Evolution of science-based uncertainty factors in noncancer risk assessment. *Regul. Toxicol. Pharmacol*. *24*, 108-120.

El-Masri, H. A., Bell, D. A., Portier, C. J. 1999. Effects of glutathione transferase theta polymorphism on the risk estimates of dichloromethane to humans. *Toxicol. Appl. Pharmacol*. *158*, 221-230.

Faustman, E. M., Ribeiro, P. L. 1990. Pharmacokinetic considerations in developmental toxicity. DANS: R.D. Hood (Ed.), *Developmental toxicology: risk assessment and the future*, Van Nostrand Reinhold, New York NY, pp. 109-136.

Fenneteau, F., Li, J., Nekka, F. 2009. Assessing drug distribution in tissues expressing P-glycoprotein using physiologically based pharmacokinetic modeling: identification of important model parameters through global sensitivity analysis. *J Pharmacokinet. Pharmacodyn. 36*, 495-522

Finster, M., Pedersen, H. 1979. Placental transfer and fetal uptake of drugs. *Br. J Anaest. 51*, 25S-28S.

Fisher, J., Mahle, D., Bankston, L., Greene, R., Gearhart, J. 1997. Lactational transfer of volatile chemicals in breast milk. *Am Ind. Hyg. Assoc. J 58*, 425-431.

Gearhart, J. M., Mahle, D. A., Greene, R. J., Seckel, C. S., Flemming, C. D., Fisher, J. W., Clewell, H. J., III. 1993. Variability of physiologically based pharmacokinetic (PBPK) model parameters and their effects on PBPK model predictions in a risk assessment for perchloroethylene (PCE). *Toxicol. Lett. 68*, 131-144.

Gentry, P. R., Clewell, H. J. 2007. Use of Physiologically Based Pharmacokinetic Modeling to Evaluate Implications of Human Variability. DANS: J. C. Lipscomb, E. Ohanian (Eds.), *Toxicokinetics and Risk Assessment*, Informa Healthcare, New York NY, pp. 211-229.

Gentry, P. R., Hack, C. E., Haber, L., Maier, A., Clewell, H. J. III. 2002. An approach for the quantitative consideration of genetic polymorphism data in chemical risk assessment: examples with warfarin and parathion. *Toxicol. Sci*. *70*, 120-139.

Gentry, P. R., Covington, T. R., Clewell, H. J. III. 2003. Evaluation of the potential impact of pharmacokinetic differences on tissue dosimetry in offspring during pregnancy and lactation. *Regul. Toxicol. Pharmacol. 38*, 1-16.

Gibaldi, M., Perrier, D. 1982. *Pharmacokinetics*. Marcel Dekker Inc, New York, NY.

Ginsberg, G., Smolenski, S., Hattis, D., Sonawane, B. 2002a. Population distribution of aldehyde dehydrogenase-2 genetic polymorphism: implications for risk assessment. *Regul. Toxicol. Pharmacol*. *36*, 297-309.

Ginsberg, G., Hattis, D., Sonawane, B., Russ, A., Banati, P., Kozlak, M., Smolenski, S., Goble, R. 2002b. Evaluation of child/adult pharmacokinetic differences from a database derived from the therapeutic drug literature. *Toxicol. Sci*. *66*, 185-200.

Ginsberg, G., Hattis, D., Sonawane, B. 2004a. Incorporating pharmacokinetic differences between children and adults in assessing children's risks to environmental toxicants. *Toxicol. Appl. Pharmacol*. *198*, 164-183.

Ginsberg, G., Hattis, D., Russ, A., Sonawane, B. 2004b. Physiologically based pharmacokinetic (PBPK) modeling of caffeine and theophylline in neonates and adults: implications for assessing children's risks from environmental agents. *J Toxicol. Environ Health A*. *67*, 297-329.

Ginsberg, G., Perkovich Foos, B., Firestone, M. 2005. Review and analysis of inhalation dosimetry methods for application to children’s risk assessment. *J Toxicol. Environ Health A*. *68*, 573-615.

Ginsberg, G., Foos, B., Dzubow, R. B., Firestone, M. 2010. Options for incorporating children's inhaled dose into human health risk assessment. *Inhal.Toxicol. 22*, 627-647.

Grant, R. L., Haney, J., Curry, A. L., Honeycutt, M. 2010. A chronic reference value for 1,3-butadiene based on an updated noncancer toxicity assessment. *J Toxicol. Environ Health B*. *13*, 460-475.

Gray, D. G. 1995. A physiologically based pharmacokinetic model for methyl mercury in the pregnant rat and fetus. *Toxicol. Appl. Pharmacol. 132*, 91-102.

Green, T. P., O'Dea, R. F., Mirkin, B. L. 1979. Determinants of drug disposition and effect in the fetus. *Annu. Rev. Pharmacol. Toxicol. 19*, 285-322.

Groen, K., Horan, M. A., Roberts, N.A., Gulati, R. S., Miljkovic, B., Jansen, E. J., Paramsothy, V., Breimer, D. D., van Bezooijen, C. F. 1993. The relationship between phenazone (antipyrine) metabolite formation and theophylline metabolism in healthy and frail elderly women. *Clin. Pharmacokinet*. *25*, 136-144.

Haber, L. 2007. Overview of approach to noncancer risk assessment. DANS: J. C. Lipscomb, E. Ohanian (Eds.), *Toxicokinetics and Risk Assessment*, Informa Healthcare, New York NY, pp. 1-25.

Haber, L., Gentry, R., Adamou, T., Krishnan, K. 2008. A framework to evaluate child-adult differences in inhalation dosimetry of gases: Application to selected systemically-acting volatile organic compounds. *The Toxicologist*.

Hack, C. E., Chiu, W. A., Jay, Z. Q., Clewell, H. J. 2006. Bayesian population analysis of a harmonized physiologically based pharmacokinetic model of trichloroethylene and its metabolites. *Regul. Toxicol. Pharmacol*. *46*, 63-83.

Haddad, S., Restieri, C., Krishnan, K. 2001. Characterization of age-related changes in body weight and organ weights from birth to adolescence in humans. *J Toxicol. Environ Health A*. *64*, 453-464.

Haddad, S., Tardif, G. C., Tardif, R. 2006. Development of physiologically based toxicokinetic models for improving the human indoor exposure assessment to water contaminants: trichloroethylene and trihalomethanes. *J Toxicol. Environ Health* A. *69*, 2095-2136.

Hakkola, J., Tanaka, E., Pelkonen, O. 1998. Developmental expression of cytochrome P450 enzymes in human liver. *Pharmacol. Toxicol. 82*, 209-217.

Hattis, D., Erdreich, L., Ballew, M. 1987. Human variability in susceptibility to toxic chemicals--a preliminary analysis of pharmacokinetic data from normal volunteers. *Risk Anal. 7*, 415-426.

Hattis, D., Banati, P., Goble, R., Burmaster, D. E. 1999a. Human interindividual variability in parameters related to health risks. *Risk Anal*. *19*, 711-726.

Hattis, D., Banati, P., Goble, R. 1999b. Distributions of individual susceptibility among humans for toxic effects. How much protection does the traditional tenfold factor provide for what fraction of which kinds of chemicals and effects? *Ann N. Y. Acad Sci*. *895:* 286-316.

Hattis, D., Ginsberg, G., Sonawane, B., Smolenski, S., Russ, A., Kozlak, M., Goble, R. 2003. Differences in pharmacokinetics between children and adults--II. Children's variability in drug elimination half-lives and in some parameters needed for physiologically-based pharmacokinetic modeling. *Risk Anal*. *23*, 117-142.

ICRP (International Commission on Radioprotection) 2002. Basic anatomical and physiological data for use in radiological protection: reference values. A report of age- and gender-related differences in the anatomical and physiological characteristics of reference individuals. ICRP Publication 89. *Ann ICRP. 32*, 5-265.

IPCS (International Programme on Chemical Safety). 1994. Environmental Health Criteria 170: Assessing human health risks of chemicals: Derivation of guidance values for health-based exposure limits. WHO/IPCS. Geneva, Switzerland.

IPCS (International Programme on Chemical Safety). 2005. Chemical-Specific Adjustment Factors (CSAFs) for Interspecies differences and human variability: Guidance Document for the Use of Data in Dose/Concentration-Response Assessment. WHO/IPCS/01.4, 1-96. Geneva, Switzerland.

IPCS (International Programme on Chemical Safety). 2006. Environmental Health Criteria 237: Principles for evaluating health risks in children associated with exposure to chemicals. WHO/IPCS. 2006. Geneva, Switzerland.

Jacqz-Aigrain, E. 2001. Pharmacologie du développement. DANS : E. Saliba, S. Hamamah, F. Gold, M. Benhamed (Eds.), *Médecine et biologie du développement, du gène au nouveau-né*, Masson, Paris, pp. 399-410.

Jensen, A. A. 1991. Transfert of chemical contaminants into human milk. DANS: A. A. Jensen, S. A. Slorach (Eds.), *Chemical contaminants in human milk*, CRC Press, Boca Raton FL, pp. 9-19.

Johnsrud, E. K., Koukouritaki, S. B., Divakaran, K., Brunengraber, L. L., Hines, R. N., and McCarver, D. G. 2003. Human hepatic CYP2E1 expression during development. *J Pharmacol. Exp. Ther*. *307*, 402-407.

Jonsson, F., Johanson, G. 2001. A Bayesian analysis of the influence of GSTT1 polymorphism on the cancer risk estimate for dichloromethane. *Toxicol. Appl. Pharmacol*. *174*, 99-112.

Jonsson, F. Johanson, G. 2002. Physiologically based modeling of the inhalation kinetics of styrene in humans using a bayesian population approach. *Toxicol. Appl. Pharmacol*. *179*, 35-49.

Kearns, G. L., Abdel-Rahman, S. M., Alander, S. W., Blowey, D. L., Leeder, J. S., Kauffman, R. E. 2003. Developmental pharmacology--drug disposition, action, and therapy in infants and children. *N. Engl. J Med. 349*, 1157-1167.

Kinirons, M. T., Crome, P. 1997. Clinical pharmacokinetic considerations in the elderly. An update. *Clin. Pharmacokinet*. *33*, 302-312.

Krauer, B., Krauer, F., Hytten, F. E. 1980. Drug disposition and pharmacokinetics in the maternal-placental-fetal unit. *Pharmacol. Ther. 10*, 301-328.

Krishnan, K. Andersen, M. E. 1993. Pharmacokinetics, individual differences. DANS: M. W. Corn (Ed.), *Handbook of hazardous materials*, Academic Press, New York NY, pp. 577-589.

Krishnan, K., Andersen, M. E. 2008. Physiologically based pharmacokinetic and toxicokinetic models. DANS: A. W. Hayes (Ed.) *Principles and Methods of Toxicology*, CRC Press, Boca Raton FL, pp. 231-292.

Krishnan, K., Johanson, G. 2005. Physiologically-based pharmacokinetic and toxicokinetic models in cancer risk assessment. *J Environ Sci. Health C. Environ Carcinog. Ecotoxicol. Rev. 23*, 31-53.

Lacroix, D., Sonnier, M., Moncion, A., Cheron, G., Cresteil, T. 1997. Expression of CYP3A in the human liver--evidence that the shift between CYP3A7 and CYP3A4 occurs immediately after birth. *Eur. J Biochem. 247*, 625-634.

Lehman, A. J. Fitzhugh, O. G. 1954. 100-fold margin of safety. *Assoc. Food Drug Off. U. S. Q. Bull. 18*, 33-35. Cité dans Price *et al.* 1999.

Liao, K. H., Tan, Y. M., Clewell, H.J. III. 2007a. Development of a screening approach to interpret human biomonitoring data on volatile organic compounds: reverse dosimetry on biomonitoring data for trichloroethylene. *Risk Anal*. *27*, 1223-1236.

Liao, K. H., Tan, Y. M., Conolly, R. B., Borghoff, S. J., Gargas, M. L., Andersen, M. E., Clewell, H.J. III. 2007b. Bayesian estimation of pharmacokinetic and pharmacodynamic parameters in a mode-of-action-based cancer risk assessment for chloroform. *Risk Anal*. *27*, 1535-1551.

Lipscomb, J. C. 2008. Categorical default approaches to uncertainty factor development. Continuing education course - Use of data for development of uncertainty factors in non-cancer risk assessment. 47th annual meeting of the Society of Toxicology. Seattle, WA. 3-16-2008.

Lipscomb, J. C., Meek, M. E., Krishnan, K., Kedderis, G. L., Clewell, H. J., Haber, L. 2004. Incorporation of pharmacokinetic and pharmacodynamic data into risk assessments. *Toxicol. Mech. Meth*. *14*, 145-158.

Lipscomb, J. C., Poet, T. S. 2008. In vitro measurements of metabolism for application in pharmacokinetic modeling. *Pharmacol. Ther. 118*, 82-103.

Lipscomb, J. C., Teuschler, L. K., Swartout, J., Popken, D., Cox, T., Kedderis, G. L. 2003. The impact of cytochrome P450 2E1-dependent metabolic variance on a risk-relevant pharmacokinetic outcome in humans. *Risk Anal*. *23*, 1221-1238.

Mahle, D. A., Gearhart, J. M., Grigsby, C. C., Mattie, D. R., Barton, H. A., Lipscomb, J. C., Cook, R.S. 2007. Age-dependent partition coefficients for a mixture of volatile organic solvents in Sprague-Dawley rats and humans. *J Toxicol. Environ Health A 70*, 1745-1751.

Malviya, S., Lerman, J., 1990. The blood/gas solubilities of sevoflurane, isoflurane, halothane and serum constituent concentrations in neonates and adults. *Anesthesiology 72*, 793-796.

Mattison, D. R. 1990. Fetal pharmacokinetic and physiological models. DANS: R. D. Hood (Ed.), *Developmental toxicology: risk assessment and the future*, Van Nostrand Reinhold, New York NY, pp. 137-154.

Mattison, D. R., Blann, E., Malek, A. 1991. Physiological alterations during pregnancy: impact on toxicokinetics. *Fundam. Appl. Toxicol. 16*, 215-218.

Meek, B., Renwick, A., Ohanian, E., Sonich-Mullin, C. 2002a. Guidance for derivation of chemical-specific adjustment factors (CSAF) - Development and implementation. *Human Ecol. Risk Assess*. *8*, 769-782.

Meek, M. E., Renwick, A., Ohanian, E., Dourson, M., Lake, B., Naumann, B. D., Vu, V. 2002b. Guidelines for application of chemical-specific adjustment factors in dose/concentration-response assessment. *Toxicology*. *181-182*, 115-120.

Mielke, H., Gundert, A., Abraham, K., Gundert-Remy, U. 2005. Acute inhalative exposure assessment: derivation of guideline levels with special regard to sensitive subpopulations and time scaling. *Toxicology. 214*, 256-267.

Miller, M. D., Marty, M. A., Arcus, A., Brown, J., Morry, D., Sandy, M. 2002. Differences between children and adults: implications for risk assessment at California EPA. *Int. J Toxicol*. *21*, 403-418.

Mörk, A. K., Johanson, G. 2010. Chemical-specific adjustment factors for intraspecies variability of acetone toxicokinetics using a probabilistic approach. *Toxicol. Sci. 116*, 336-348.

Naumann, B. D., Silverman, K. C., Dixit, R., Faria, E. C., Sargent, E. V. 2001. Case studies for catergorical data-derived adjustment factors. *Human Ecol. Risk Assess*. *7*, 61-105.

Naumann, B. D., Dolan, D. G., Sargent, E. V. 2004. Rationale for the chemical-specific adjustment factors used to derive an occupational exposure limit for timolol maleate. *Human Ecol. Risk Assess*. *10*, 1-13.

Neafsey, P., Ginsberg, G., Hattis, D., Johns, D. O., Guyton, K. Z., Sonawane, B. 2009. Genetic Polymorphism in CYP2E1: Population Distribution of CYP2E1 Activity. *J Toxicol. Environ Health B. 12*, 362-388.

Nong, A., McCarver, D. G., Hines, R. N., Krishnan, K. 2006. Modeling interchild differences in pharmacokinetics on the basis of subject-specific data on physiology and hepatic CYP2E1 levels: a case study with toluene. *Toxicol. Appl. Pharmacol*. *214*, 78-87.

Nong, A., Krishnan, K. 2007. Estimation of interindividual pharmacokinetic variability factor for inhaled volatile organic chemicals using a probability-bounds approach. *Regul. Toxicol. Pharmacol*.

NRC (National Research Council). 1993. Special characteristics of children. DANS: National Research Council (U.S.) and Committee on Pesticides in the Diets of Infants and

Children (Eds), *Pesticides in the diets of infants and children*, National Academy Press. Washington DC, pp. 23-48.

NRC (National Research Council). (1983). Risk Assessment in the federal government: managing the process. National Academy Press, Washington DC., 192 p.

Palmer, R. B., Brent, J. 2005. Derivation of a chemical-specific adjustment factor (CSAF) for use in the assessment of risk from chronic exposure to ethylene glycol: Application of international programme for chemical safety guidelines. *Toxicol. Appl. Pharmacol. 207*, 576-584.

Pelekis, M., Krewski, D., Krishnan, K. 1997. Physiologically based algebraic expressions for predicting steady-state toxicokinetics of inhaled vapors. *Toxicol. Meth. 7*, 205-225.

Pelekis, M., Gephart, L. A., Lerman, S. E. 2001. Physiological-model-based derivation of the adult and child pharmacokinetic intraspecies uncertainty factors for volatile organic compounds. *Regul. Toxicol. Pharmacol*. *33*, 12-20.

Pelekis, M., Nicolich, M. J., Gauthier, J. S. 2003. Probabilistic framework for the estimation of the adult and child toxicokinetic intraspecies uncertainty factors. *Risk Anal*. *23*, 1239-1255.

Price, K., Haddad, S., Krishnan, K. 2003a. Physiological modeling of age-specific changes in the pharmacokinetics of organic chemicals in children. *J Toxicol. Environ Health A*. *66*, 417-433.

Price, P. S., Conolly, R. B., Chaisson, C. F., Gross, E. A., Young, J. S., Mathis, E. T., Tedder, D. R. 2003b. Modeling interindividual variation in physiological factors used in PBPK models of humans. *Crit. Rev Toxicol*. *33*, 469-503.

Price, P. S., Keenan, R. S., Schwab, B. 1999. Defining the interindividual (intraspecies) uncertainty factor. *Human Ecol. Risk Assess*. *5*, 1023-1033.

Ragas, A. M., Brouwer, F. P., Buchner, F. L., Hendriks, H. W., Huijbregts, M. A. 2009. Separation of uncertainty and interindividual variability in human exposure modeling. *J Expo. Sci. Environ Epidemiol. 19*, 201-212.

Reed, K. J., Jiménez, M., Freeman, N. C., Lioy, P. J. 1999. Quantification of children's hand and mouthing activities through a videotaping methodology. *J Expo. Anal. Environ Epidemiol*. *9*, 513-520.

Renwick, A. G. 1993. Data-derived safety factors for the evaluation of food additives and environmental contaminants. *Food Addit. Contam*. *10*, 275-305.

Renwick, A. G., Lazarus, N. R. 1998. Human variability and noncancer risk assessment--an analysis of the default uncertainty factor. *Regul. Toxicol. Pharmacol*. *27*, 3-20.

Renwick, A. G. 1998. Toxicokinetics in infants and children in relation to the ADI and TDI. *Food Addit. Contam 15 Suppl*, 17-35.

Renwick, A. G., Dorne,J. L., Walton, K. 2000. An analysis of the need for an additional uncertainty factor for infants and children. *Regul. Toxicol. Pharmacol*. *31*, 286-296.

Renwick, A. G., Dorne, J. L., Walton, K. 2001. Pathway-related factors: The potential for human data to improve the scientific basis of risk assessment. *Human Ecol. Risk Assess*. *7*, 165-180.

Ritter, L., Totman, C., Krishnan, K., Carrier, R., Vézina, A., Morisset, V. 2007. Deriving uncertainty factors for threshold chemical contaminants in drinking water. *J Toxicol. Environ Health B*. *10*, 527-557.

Ronis, M. J. J., Lindros, K. O., Ingelman-Sundberg, M. 1996. The CYP2E family. In C. Ioannides (Ed.), *Cytochrome P450 Metabolic and Toxicological Aspects*, CRC PRess, Boca Raton FL, pp. 211-240.

SA (sans auteur). 2002. A national and international debate on default uncertainty factors vs data-derived uncertainty factors. *Human Ecol. Risk Assess*. *8*, 895-911.

Sarangapani, R., Gentry, P. R., Covington, T. R., Teeguarden, J. G., Clewell, H. J., III. 2003. Evaluation of the potential impact of age- and gender-specific lung morphology and ventilation rate on the dosimetry of vapors. *Inhal. Toxicol. 15*, 987-1016.

Schmucker, D. L., Lonergan, E.T. 1987. Aging and drug disposition. *Rev. Biol. Res. Aging 3*, 509-535.

Schmucker, D. L. 2001. Liver function and phase I drug metabolism in the elderly: a paradox. *Drugs Aging*. *18*, 837-851.

Shimada, T., Yamazaki, H., Mimura, M., Inui, Y., Guengerich, F. P. 1994. Interindividual variations in human liver cytochrome P-450 enzymes involved in the oxidation of drugs, carcinogens and toxic chemicals: studies with liver microsomes of 30 Japanese and 30 Caucasians. *J Pharmacol. Exp. Ther. 270*, 414-423.

Sidhu, K. S. 1991. Standard setting processes and regulations for environmental contaminants in drinking water: state versus federal needs and viewpoints. *Regul. Toxicol. Pharmacol*. *13*, 293-308.

Silverman, K. C., Naumann, B. D., Holder, D. J., Dixit, R., Faria, E. C., Sargent, E. V., Gallo, M. A. 1999. Establishing data-derived adjustment factors from published pharmaceutical clinical trial data. *Human Ecol. Risk Assess*. *5*, 1059-1089.

Simon, T. W. 1997. Combining physiologically based pharmacokinetic modeling with Monte Carlo simulation to derive an acute inhalation guidance value for trichloroethylene. *Regul. Toxicol.Pharmacol*. *26*, 257-270.

Simon, T. W. 1999. Two-dimensional Monte Carlo simulation and beyond: A comparison of several probabilistic risk assessment. *Human Ecol. Risk Assess. 5*, 823-843.

Skowronski, G. A. Abdel-Rahman, M. S. 2001. Relevance of the 10X uncertainty factor to the risk assessment of drugs used by children and geriatrics. *Human Ecol. Risk Assess*. *7*, 139-152.

Sonnier, M., Cresteil, T. 1998. Delayed ontogenesis of CYP1A2 in the human liver. *Eur. J Biochem. 251*, 893-898.

Stern, A. H. 1997. Estimation of the interindividual variability in the one-compartment pharmacokinetic model for methylmercury: implications for the derivation of a reference dose. *Regul. Toxicol.Pharmacol*. *25*, 277-288. Cité dans Stern *et al.* (2002).

Stern, A. H., Clewell, H. J., Swartout, J. 2002. An objective uncertainty factor adjustment for methylmercury pharmacokinetic variability. *Human Ecol. Risk Assess*. *8*, 885-894.

Suh, D. H., Abdel-Rahman, M. S. 2002. Evaluating the 10X uncertainty factor for children and elderly with data-derived values for neuromuscular blocking agents. *Human Ecol. Risk Assess*. *8*, 841-858.

Swartout, J., Rice, G. 2000. Uncertainty analysis of the estimated ingestion rates used to derive the methylmercury reference dose. *Drug Chem. Toxicol*. *23*, 293-306. Cité dans Stern *et al.* 2002.

Sweeney, L. M., Gargas, M. L., Strother, D. E., Kedderis, G. L. 2003. Physiologically based pharmacokinetic model parameter estimation and sensitivity and variability analyses for acrylonitrile disposition in humans. *Toxicol. Sci. 71*, 27-40.

Tan, Y.M., Liao, K. H., Clewell, H. J. III. 2007. Reverse dosimetry: interpreting trihalomethanes biomonitoring data using physiologically based pharmacokinetic modeling. *J Expo. Sci. Environ. Epidemiol*. *17*, 591-603.

Tardif, R., Droz, P. O., Charest-Tardif, G., Pierrehumbert, G., Truchon, G. 2002. Impact of human variability on the biological monitoring of exposure to toluene: I. Physiologically based toxicokinetic modelling. *Toxicol. Lett*. *134*, 155-163.

Thomas, R. S., Bigelow, P. L., Keefe, T. J., Yang, R. S. 1996. Variability in biological exposure indices using physiologically based pharmacokinetic modeling and Monte Carlo simulation. *Am. Ind. Hyg. Assoc*. *J 57*, 23-32.

Timchalk, C., Kousba, A., Poet, T.S. 2002. Monte Carlo analysis of the human chlorpyrifos-oxonase (PON1) polymorphism using a physiologically based pharmacokinetic and pharmacodynamic (PBPK/PD) model. *Toxicol. Lett. 135*, 51-59.

U.S. EPA (U.S. Environmental Protection Agency). 1997a. Exposure factors handbook. U.S. Environmental Protection Agency, Office of Research and Development National Center for Environmental Assessment, Washington DC.

U.S. EPA (U.S. Environmental Protection Agency). 1997b. Guideline principles for Monte Carlo Analysis. Risk Assessment forum, document no. EPA/630/R-97/001. U.S. Environmental Protection Agency, Washington DC.

U.S. EPA (U.S. Environmental Protection Agency). 2001. Risk Assessment Guidance for Superfund: Volume III - Part A, Process for Conducting Probabilistic Risk Assessment. EPA 540-R-02-002. Washington, DC, Office of Emergency and Remedial Response.

U.S. EPA (U.S. Environmental Protection Agency). 2002 A review of the reference dose and reference concentration process. U.S. Environmental Protection Agency. Risk Assessment Forum. EPA/630/P-02/00F. Washington DC.

U.S. EPA (U.S. Environmental Protection Agency). 2005. Guidelines for Carcinogen Risk Assessment. EPA/630/P-03/001F. Washington DC, U.S. Environmental Protection Agency.

Valcke, M., Krishnan, K. 2009. Physiologically Based Pharmacokinetic Modeling in the Risk assessment of Developmental Toxicants. DANS: D. K. Hansen, B. D. Abbott (Eds.), *Developmental Toxicology*. Informa Health Care, New York, NY pp. 243-274.

Walker, K., Hattis, D., Russ, A., Sonawane, B., Ginsberg, G. 2007. Approaches to acrylamide physiologically based toxicokinetic modeling for exploring child-adult dosimetry differences. *J Toxicol. Environ. Health A*. *70*, 2033-2055.

Weisel, C. P., Jo, W. K. 1996. Ingestion, inhalation, and dermal exposures to chloroform and trichloroethene from tap water. *Environ Health Perspect. 104*, 48-51.

Yang, Y., Georgopoulos, P. G. 2010. A bayesian population PBPK model for multiroute chloroform exposure. *J Exp. Sci. Environ. Epi. 20*, 326-341

Yokley, K., Tran, H. T., Pekari, K., Rappaport, S., Riihimaki, V., Rothman, N., Waidyanatha, S., Schlosser, P. M. 2006. Physiologically-based pharmacokinetic modeling of benzene in humans: a Bayesian approach. *Risk Anal*. *26*, 925-943.

Yoon, M., Madden, M. C., Barton, H. A. 2007. Extrahepatic metabolism by CYP2E1 in PBPK modeling of lipophilic volatile organic chemicals: impacts on metabolic parameter estimation and prediction of dose metrics. *J Toxicol. Environ Health A. 70*, 1527-1541.

Zeeh, J., Platt, D. 2002. The aging liver: structural and functional changes and their consequences for drug treatment in old age. *Gerontology*. *48*, 121-127.

Zhao, Q., Unrine, J., Dourson, M. 1999. Replacing the default values of 10 with data-derived values: A comparison of two different data-derived uncertainty factors for boron. *Human Ecol. Risk Assess*. *5*, 973-989.

Annexe : Autres contributions réalisées durant et en lien avec les études doctorales

PUBLICATIONS

ARTICLE PUBLIÉ

1- **Valcke M**, Krishnan K (2010). An Assessment of the Interindividual Variability of Internal Dosimetry during Multi-Route Exposure to Drinking Water Contaminants*. International Journal of Environmental research and Public Health*. Vol. 7(11), 4002-4022. doi.: 10.3390/ijerph7114002.

CHAPITRES DE LIVRES

2- **Valcke M**, Krishnan K (2009) Physiologically-Based Pharmacokinetic models in cancer risk assessment Ch. 21 Dans: CH Hsu, T Stedeford (eds). *Cancer Risk Assessment*. Jon Wiley & Sons. Hoboken NJ.

3- **Valcke M**, Krishnan K (2009) Physiologically-Based Pharmacokinetic modeling in the risk assessment of developmental toxicants. Ch. 9 Dans: DK Hansen, BD Abbott (eds). *Developmental Toxicology 3rd ed*. Informa Health Care Publishers. New York.

EXPOSÉS LORS DE CONGRÈS, SYMPOSIUMS, CONFÉRENCES

1- **Valcke M**. Krishnan K (2011). *Evaluation of the impact of demography on the adequacy of the human kinetic adjustment factor (HKAF).* Annual meeting of the Society of Toxicology, Washington, D.C., mars 2011.

2- Krishnan K. **Valcke M**. (2011). *An assessment of the impact of exposure duration and intensity on the human kinetic adjustment factor (HKAF).* Annual meeting of the Society of Toxicology, Washington, D.C., mars 2011.

3- **Valcke M**. Krishnan K (2010). *Are sensitive subpopulations better protected by defining the uncertainty factor based on the finite sample size model or the sensitive subpopulation model?* Colloque annuel de la Société de Toxicologie du Canada, Montréal, décembre 2010.

4- **Valcke M**. Krishnan K (2010). Étude de la variabilité interindividuelle de l'exposition multivoie aux contaminants de l'eau potable. Présentation faite au colloque annuel du Réseau de recherche en santé environnementale du Québec, Institut Armand Frappier, Laval, Québec, juin 2010.

5- **Valcke M**. Krishnan K (2010). *Evaluation of the magnitude of toxicokinetic interindividual variability factor (IVF-TK): impact of subpopulations and chemical characteristics*- Annual meeting of the Society of Toxicology, Salt Lake City, Utah, mars 2010.

6- **Valcke M**. Krishnan K (2009). Validation d'un algorythme toxicocinétique pour caractériser la variabilité interindividuelle des doses internes suite à l'exposition chronique aux contaminants de l'eau potable. Affiche présentée au colloque

annuel du Réseau de recherche en santé environnementale du Québec, Institut Armand Frappier, Laval, Québec, juin 2009.

7- **Valcke M**. Krishnan K (2009). *Evaluation of the magnitude of the interindividual variability factor (ivf) for cyp2e1 and adh substrates as a function of chemical and subpopulations characteristics*. Colloque annuel de la Société de Toxicologie du Canada, Montréal, décembre 2009.

8- **Valcke M**. Krishnan K (2009). *An assessment of the impact of exposure route on the interindividual variability factor (IVF) for drinking water contaminants (DWCs)*- Annual meeting of the Society of Toxicology, Baltimore, Maryland, mars 2010.

9- **Valcke M**. Krishnan K (2008). *An assessment of the interindividual variability factor applicable to multi-route exposure to drinking water contaminants*. Colloque annuel de la Société de Toxicologie du Canada, Montréal, décembre 2008.

10- **Valcke M**. Krishnan K (2008) Évaluation par une approche populationnelle du facteur d'incertitude interindividuelle toxicocinétique utilisé en analyse du risque: le cas du trichloroéthylène. Rencontre annuelle du Réseau de Recherche en Santé Environnementale, Institut Armand Frappier, mai 2008

11- **Valcke M**. Krishnan K (2008). *Assessing the adequacy of the default interindividual variability factor (IVF) using a physiologically-based steady-state (PBSS) algorithm*"- Annual meeting of the Society of Toxicology, Seattle, Washington, mars 2008.

BOURSES ET DISTINCTIONS

2011: - "John Doull Award for an outstanding presentation in Risk Assessment by a student or post-doctoral scientist", Congrès de la *Society of Toxicology (SOT)* des États-Unis, Washington, D.C.

- Bourse de voyage du Réseau de Recherche en santé environnementale du Québec. Destination: Congrès de la *SOT* des États-Unis, Washington, D.C.

2010: - "Graduate student travel award", Congrès de la *SOT* des États-Unis, Salt Lake City, Utah.

- "Blue ribbon award for top 10 % abstract in risk assessment" Congrès de la *SOT* des États-Unis, Salt Lake City, Utah.

2009: - Prix (bourse) de la meilleure affiche, colloque du Réseau de recherche en santé environnementale du Québec, Institut Armand Frappier, Laval, Québec. Juin 2009.

- "Perry J Gehring Risk Assessment SS Best Student Abstract Award", Congrès de la *SOT* des États-Unis, Baltimore, Maryland.

2008: - "Blue ribbon award for top 10 % abstract in risk assessment ", Congrès de la *SOT* des États-Unis, Seattle, Washington